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CONTENTS

PART 1—JANUARY 1957

PART 2—APRIL 1957

PART 3—JULY 1957

PART 4—OCTOBER 1957

FERTILIZATION, EARLY CLEAVAGE AND ASSOCIATED PHENOMENA IN THE FIELD VOLE (*MICROTUS AGRESTIS*)

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INTRODUCTION

The reproductive processes of the field vole were studied by Brambell & Hall (1939) who presented data on a number of features including breeding season, oestrous cycle, mean numbers of eggs ovulated and of embryos *in utero*, and sex ratio. Their material consisted of animals captured in the wild state. From 1930 to 1940 Ranson (1947) succeeded in breeding voles in the laboratory at the Bureau of Animal Population at Oxford. A colony, that has been augmented at intervals by further captures in the field, is maintained there at the present time. Early in 1955 some animals from this source were brought to the National Institute for Medical Research to start a new colony.

A special point of interest with *Microtus agrestis* lies in the remarkably large size of the sex chromosomes, relative to the autosomes, as first noted by Matthey (1949). The form and behaviour of the sex chromosomes in spermatogenesis have been described in detail (Matthey, 1950). At spermatogonial metaphase the X-chromosome was found to be about $7{\cdot}5\,\mu$ long and the Y-chromosome about $5{\cdot}3\,\mu$ long, while the autosomes did not exceed $3\,\mu$ in length. A few other animals, such as the three species of hamster (Matthey, 1952) and *M. oeconomus* (Muldal, 1948), also display sex chromosomes that are larger than the autosomes, but the disparity is seldom as striking as in *M. agrestis*.

The present report deals with investigations made on processes associated with fertilization and on the appearance of the sex chromosomes at various stages. Collaborative studies on the oestrous cycle and mating behaviour have also been made but are to be described in a separate communication (Chitty & Austin, manuscript in preparation).

METHODS

During the period of observation the voles were kept in groups of up to a dozen animals in mouse cages. The conditions were similar to those under which experimental mice are maintained, except that granulated peat and a little hay were used for bedding in place of sawdust. Under these circumstances the voles experience periods of continuous oestrus during which coitus may occur at any time and induce ovulation (Chitty & Austin, manuscript in preparation).

The occurrence of coitus was mostly known from the presence of spermatozoa in the vaginal smear—a copulation plug was seen on only a few occasions.

Eggs were recovered by dissection of the Fallopian tubes or the uteri under normal saline solution and were then transferred to a slide and covered with a

cover-slip with vaselined edges. As a routine, the eggs were examined by means of a phase-contrast microscope, first in an uncompressed state and then compressed between cover-slip and slide. If chromosomes were present the eggs were fixed with a solution of 5 % acetic acid in absolute alcohol drawn under the cover-slip and stained by the same procedure with a 1 % aqueous solution of toluidine blue. After this it was possible to exert a little more pressure on the cover-slip and thus to improve somewhat the spread of the chromosomes. When such a chromosome preparation was examined it was often found to be better seen with phase-contrast than with direct illumination.

Testis-squash preparations of chromosomes were made after fixation with acetocarmine solution; they were examined by phase-contrast or ultra-violet microscopy.

Diameters of eggs, nuclei and nucleoli, and the dimensions of spermatozoa were measured with an eyepiece micrometer and the vertical depth of nuclei by means of the fine focusing adjustment on the microscope. The number of spermatozoa at the site of fertilization was determined in the manner previously described (Austin, 1952*a*).

OBSERVATIONS

(1) *The gametes*

In this paper the spermatozoon is considered to be made up of head and tail, and the latter to consist of neck, mid-piece, main-piece and end-piece, as defined by Austin & Bishop (1957). The filiform end-piece was difficult to see in vole spermatozoa and was ignored in this investigation. The head of the vole spermatozoon is of the hooked type characteristic of many murine rodents, but the apex of the head is more strongly recurved than usual (Pl. 1, fig. 1). Measurements made on ten spermatozoa showed the mean length of the whole spermatozoon to be $103{\cdot}5\,\mu$, the head $6{\cdot}9\,\mu$ and the neck and mid-piece $27{\cdot}4\,\mu$. The mid-piece is quite smooth in appearance and only a little thicker than the tail.

Observations were made on a total of 282 eggs from 76 voles.

Twenty-one eggs obtained from follicles as primary or secondary oocytes had a finely granular cytoplasm containing a number of small irregular masses of coarser granular material. The appearance was similar to that of the pronucleate egg (Pl. 1, fig. 9). Later on, during the cleavage stages, the blastomeres have a number of refractile plaque-like bodies on, or near, the surface which look like flattened fat droplets (Pl. 3, figs. 26, 27). Neither the oocyte nor the recently ovulated unpenetrated egg show cortical granules such as those described in hamster eggs (Austin, 1956*b*). As in other animals, the vole primary oocyte before maturation has a large spherical nucleus containing a single nucleolus. The mean nuclear volume in six oocytes was found to be $5200\,\mu^3$, and the nucleolar volume $94\,\mu^3$. The ovulated unpenetrated egg has a distinct perivitelline space and the zona pellucida lacks obvious structure. A cumulus mass surrounds the freshly ovulated egg; it is similar to but not quite so voluminous as that around rat and mouse eggs.

From the Fallopian tubes of 42 voles a total of 173 1- and 2-cell eggs was recovered under conditions in which loss was considered unlikely to have occurred. The mean number of eggs per vole was thus 4·1. This rather low figure may be

owing in part to the fact that most of the animals used were young and nulliparous. The mean overall diameter of 15 freshly ovulated unpenetrated eggs was found to be 99·8 μ (range 92·5–106·2 μ), the mean vitelline diameter was 63·6 μ (range 53·8–68·8 μ), and the thickness of the zona was 10·4 μ (range 10·0–12·5 μ). Similar measurements on 15 pronucleate eggs gave the mean overall diameter as 95·9 μ (range 87·5–107·5 μ), the mean vitelline diameter as 59·7 μ (range 53·8–66·3 μ) and the thickness of the zona as 10·0 μ (range 8·8–11·3 μ). Differences between the corresponding measurements of penetrated and unpenetrated eggs were not significant.

The solubility of the zona pellucida in dilute acid solution was tested with mixtures of B.D.H. buffer solutions. The zona was rapidly removed from 26 eggs in a solution of pH 2·4. Of 24 eggs tested with a solution of pH 2·7, 8 eggs lost the zona and 16 eggs retained it for at least 1 hr. Solutions of pH 3·0–7·0 had no obvious effect upon the zona.

Of 16 unpenetrated eggs recovered from 6 voles within 24 hr. after coitus all had one polar body and exhibited the metaphase of the second maturation division. In fixed eggs the spindles were usually clearly defined, and the shape suggested that the constituent fibres were brought to a point at each pole (Pl. 1, fig. 5). A total of 21 unfertilized eggs was obtained from 8 voles 36–40 hr. after coitus; 5 of these still exhibited the second maturation spindle in metaphase; three showed chromosome scatter; in one egg a single large nucleus had developed (Pl. 2, fig. 12), and in the remaining 12 eggs nothing distinctive was observed.

(2) *Fertilization*

The number of spermatozoa in the ampullary region of the Fallopian tube, which seems to be the site of fertilization in this animal, was determined in 8 voles. The mean figure obtained was 124 spermatozoa, range 28–360.

A total of 238 eggs was recovered from 67 voles that had mated; of these, 195 eggs (82 %) from 53 voles were undergoing fertilization or early cleavage. Only four of the animals yielded both penetrated and unpenetrated eggs, so that the rate of fertilization in the eggs recovered from the remaining 49 voles was 100 %. Of 85 eggs undergoing fertilization or in the 2-cell stage and recovered within 48 hr. after coitus, 9 eggs had one polar body, 70 had 2 polar bodies and 6 had 3 polar bodies. In several penetrated eggs, especially the ones with a single polar body, the perivitelline space also contained debris that could have been formed by the fragmentation of a polar body. Sometimes, in 2-cell eggs, rounded or oddly shaped masses were seen, often only a little smaller than a polar body. These might presumably represent the products of deutoplasmolysis.

The mid-piece of the fertilizing spermatozoon shows evidence of disintegration during the period of pronuclear growth (Pl. 2, fig. 10). Generally nothing could be found of the mid-piece or main-piece in the cytoplasm of cleaved eggs, but occasionally a thread-like object, supposedly the axial element, was observed in one blastomere of 2-cell eggs.

An unexpected early observation was that in many eggs it was apparently only the head of the spermatozoon that entered the vitellus, the mid-piece and main-piece of the tail remaining within the perivitelline space (Pl. 1, fig. 8). Before this

finding could be accepted it was necessary to eliminate three other explanations: first, that the observed object in the perivitelline space was a supplementary spermatozoon with head detached; secondly, that it was only the main-piece that had failed to pass into the vitellus; thirdly, that the entry of the whole tail had merely been delayed. The first possibility could be ruled out because in none of the eggs had a spermatozoon head been seen in the perivitelline space. Moreover, the spermatozoon mid-piece and main-piece can readily be distinguished in the undivided egg when they do enter the vitellus, and in no egg have these structures been seen in both vitellus and perivitelline space. The second possibility could be eliminated because, upon close study, the mid-piece could be recognized with assurance in many undivided eggs (Pl. 2, figs. 13, 14). The third possibility could be rejected because the whole tail was recognizable in 2-cell eggs (Pl. 2, fig. 15; Pl. 3, fig. 21). In later stages a similar structure can be found (Pl. 3, figs. 26, 29), but, as it is now in rather a ravelled state and apparently reduced in size, uncertainty exists that the mid-piece is still present. The granular appearance of the mid-piece as early as the 2-cell stage (Pl. 2, fig. 15) suggests that it is undergoing some kind of change.

Among a total of 195 eggs that were undergoing fertilization or early cleavage the spermatozoon tail had evidently failed to follow the head into the vitellus in 88 eggs (45 %). In none of these eggs was there otherwise any indication of abnormality.

In none of the 195 penetrated eggs was there a supplementary spermatozoon. Only one instance of polyspermy was seen—this egg had three pronuclei and two spermatozoon tails within the cytoplasm. It was otherwise normal. Since the spermatozoon tail lying in the egg cytoplasm is rarely distinguishable in the cleavage stages, polyspermy could only have been recognized with certainty in the 57 pronucleate eggs and in two eggs in which the prophase cleavage chromosomes were still separated. The incidence of polyspermy was thus about 2 %.

Table 1. *Mean pronuclear and total nucleolar volumes, and mean number of nucleoli, in the pronuclei of* Microtus agrestis *eggs*

No. of eggs	Pronuclear volume: Larger pronuclei	Pronuclear volume: Smaller pronuclei	Total nucleolar volume: Larger pronuclei	Total nucleolar volume: Smaller pronuclei	No. of nucleoli: Larger pronuclei	No. of nucleoli: Smaller pronuclei
10	$2013\mu^3$	$1594\mu^3$	$116\mu^3$	$105\mu^3$	4·9	5·6
(range)	(1664–2506)	(1361–1790)	(81–149)	(66–180)	(1–11)	(1–11)

The pronuclei were similar in appearance to those in rat, mouse and hamster eggs, each pronucleus having one or, more commonly, several nucleoli. Generally, one pronucleus was larger than the other—this may be the male pronucleus because the male is the larger pronucleus in rat and mouse eggs and because in the polyspermic vole egg seen two of the pronuclei were larger than the third. In some of the vole eggs, however, the pronuclei were of almost the same size. Measurements of pronuclei were made in 10 eggs that were at the stage of full pronuclear development, and the calculated volumes of pronuclei and nucleoli are recorded in Table 1. Mean pronuclear volume was found to be $2013\mu^3$ for the larger pronuclei and $1594\mu^3$ for the smaller.

Total nucleolar volumes were $116\mu^3$ and $105\mu^3$, respectively. The number of nucleoli varied from 1 to 11 in both pronuclei, the mean number being about 5.

Often the larger nucleoli in pronuclei were found to contain a spherical body of bright contrast which seemed to be fluid in nature. In several eggs a smaller spherical body of medium contrast was observed within the other inclusion, the whole giving a most unusual appearance, reminiscent of an archery target (Pl. 2, fig. 11).

(3) *Cleavage and transport*

Seven female voles were found to be mated by 10 a.m. after having been placed with males at 9 a.m. These animals were killed between 9 and 10 a.m. the next day. Of the 31 eggs recovered, 8 were pronucleate, 14 exhibited stages in the formation and division of the first cleavage spindle, and 9 were in the 2-cell stage. All voles provided eggs that fell into at least two of these categories. The first cleavage of the vole egg can therefore be said to occur about 24 hr. after coitus. The times of the later cleavages are difficult to assess owing to the variations in the stages shown by eggs recovered at different intervals after coitus (Table 2). Thus 7 voles killed approximately 40 hr. after coitus yielded 2-, 3- and 4-cell eggs, 6 voles killed about 50 hr. after coitus provided eggs in different cleavage stages from the 2-cell to the morula of 16–20 cells, and 6 voles killed at 70 hr. after coitus gave eggs ranging from 8-cell to blastocysts. Assuming the blastocyst to be composed of 32 cells, the maximum rate of cleavage must be about once every 12 hr. on the average.

Table 2. *Number, stage and position of* Microtus agrestis *eggs recovered at various times after coitus*

Hours after coitus	No. of voles	Eggs from tube		Eggs from uterus	
		No.	Stages	No.	Stages
24	7	31	Pn., Syn., 2	0	—
40	7	26	2, 3, 4,	0	—
50	6	16	2, 3, 4, 16	4	4, 5, 6, M
70	6	10	8, 16, M, B	7	8, 16, M, B

Pn., pronuclear stage; Syn., syngamy; M, morula; B, blastocyst.
The numbers in the 'stages' columns refer to the number of blastomeres composing the eggs.

The data in Table 2 also testify to the variability of the rate of passage of eggs into the uterus. The first eggs evidently passed out of the Fallopian tube between 40 and 50 hr. after coitus, even though some were still in the 4- to 6-cell stages (Pl. 3, fig. 26). By 70 hr. after coitus more eggs had passed into the uterus, but the majority, including an early blastocyst (Pl. 3, fig. 30), were still in the Fallopian tube.

(4) *Chromosomes*

The distinctive appearance of the sex chromosomes in *Microtus agrestis* is well shown by the ultra-violet photomicrograph (Pl. 1, fig. 2) which depicts a spermatogonial metaphase. There is a suggestion in the chromosomes of a spiral structure and the characteristic V-form of the X-chromosome is evident. The more distinct constriction in the shorter arm of the X-chromosome is the centromere, and there

is also another constriction nearer the end of the chromosome. The *Y*-chromosome has no noticeable constriction, which is in conformity with an acro- or telocentric disposition of the centromere. In the first meiotic metaphase shown in Pl. 1, fig. 3, the sex chromosomes, now paired, lie somewhat removed from the group of autosomes—probably this degree of separation is artefactual. It was found that, in the preparations studied, meiotic chromosomes were never as clearly defined as mitotic chromosomes. *X*- and *Y*-chromosomes could also be identified in meta-, ana- and telophase stages of the second meiosis, but the definition was poor. First and second maturation spindles were also studied in oocytes recovered from follicles. The chromosomes could be seen in living eggs but were more easily photographed after fixation and staining. The *X*-chromosomes were found to occupy a conspicuous peripheral position on the spindle (Pl. 1, fig. 4) and the two *X*-chromatids were similarly disposed (Pl. 1, fig. 6). Even the chromatids displayed the bent shape characteristic of the *X*-chromosome.

Altogether about 30 eggs have been examined that presented various stages leading up to the formation of the first cleavage spindle and including the mitosis. The chromosomes are very large at this time and the metaphase plate extends well beyond the bounds of the spindle (Pl. 2, fig. 16). In a particularly favourable preparation (Pl. 2, fig. 18) the *X*-chromosome could be measured fairly accurately and was found to be 24μ in length. Recognition of *X*- and *Y*-chromosomes was generally possible but was easier at metaphase than at the prophase stages or at telophase. Much of the difficulty was probably due to the method used in making the preparations. Since it was necessary first to examine the eggs in the living condition the circumstances were not ideal for making chromosome squashes. Nearly always the sex chromosomes, whether two *X*'s or an *X* and a *Y*, did not lie together on the spindle and sometimes occupied opposite sides of the group. This was true also of the second cleavage spindle, as shown in Pl. 3, figs. 22 and 24.

DISCUSSION

The previously recognized smallest mammalian eggs are those of the rat and mouse, which have mean vitelline diameters of $70–75\mu$ (Hartman, 1929). The vole egg, with a vitellus of about 60μ in diameter, is of a distinctly smaller size. The overall diameter is also less, being about 96μ, as compared with $115–120\mu$ in the other species mentioned. Consistently, the pronuclei at full development are smaller too. Thus, while the sum of the male and female pronuclear volumes is about $8000\mu^3$ in the rat (Austin, 1952*b*), $5800\mu^3$ in the mouse (Austin & Braden, 1955), and $5700\mu^3$ in the hamster (Austin, 1956*a*), it is only $3600\mu^3$ in the vole. The figures obtained for the sum of the total nucleolar volumes in these animals show a similar trend: 770, 500, 340 and $220\mu^3$, respectively. The disparity between male and female pronuclei, so evident in the rat and mouse, in which the male pronucleus is about twice the size of the female pronucleus, is not so pronounced in the vole, in which the larger pronucleus is only 25% larger than the smaller, but more so than in the hamster, in which the pronuclei differ by only about 10%. The two concentric nucleolar inclusions are a curious feature of some vole eggs. The larger inclusion is of a type that has often been seen in other mammalian eggs; it is probably nucleoplasmic in nature and may signify the synthesis of nucleoplasm within the nucleolus

(Izquierdo, 1955). The inner inclusion does not seem to have been observed in other species—it may perhaps be nucleolar material.

From available evidence it seems to be a general rule in animals that the entire spermatozoon enters the egg at the commencement of fertilization (Wilson, 1928). Among invertebrates, *Nereis* appears to be the sole exception; in this species the whole tail is regularly left outside the egg. Sometimes the main-piece fails to enter the egg in sea urchins. Entry of the entire spermatozoon has been established for most of the mammals that have been thoroughly investigated (see Gresson, 1941, 1951), though doubt has been expressed that this always occurs. The vole seems to be the first mammal reported in which exclusion of both mid-piece and main-piece is a common and clearly demonstrable occurrence—the actual incidence observed among penetrated eggs was 45 %. Whilst the main-piece of the spermatozoon may well be concerned only with motility, the significance of the mid-piece is that it can represent the chief cytoplasmic contribution of the male to the future embryo and thus constitute the mechanism for cytoplasmic inheritance (Wilson, 1928; Brambell, 1930). It is therefore of considerable interest that early development can proceed in an apparently normal manner without the participation of the spermatozoon mid-piece. On the assumption that exclusion of the tail occurs with a similar frequency in animals living under natural conditions, the data of Brambell & Hall (1939) indicate that later development also is probably unimpeded. They found in their field material that the mean number of eggs ovulated was 5·5 and the mean number of embryos *in utero* was 5·0. A further small loss is suggested by the mean number of nest young, namely 4·8, recorded in earlier work cited by Brambell & Hall. The total loss was thus only about 13 %, which is reasonably accounted for as due to failure of fertilization—in the present series about 18 % of eggs from mated voles were unfertilized.

It is, of course, uncertain that the mid-piece within the perivitelline space need be quite without influence on the embryo. Observations suggested that dissolution may occur here, and it is at least conceivable that the products have some significance for the embryo. The possibility of dissolution within the perivitelline space is implied by Lams & Doorme's (1908) remark that supplementary spermatozoa in the mouse egg seem to disappear during the cleavage stages.

The early disintegration of the spermatozoon mid-piece, when it does enter the vitellus, means that its component elements, notably mitochondria, may be equally distributed between the two blastomeres at the first cleavage. This happens in the mouse egg (Gresson, 1941), but in the rat egg the mitochondria are released chiefly after the first cleavage (Blandau & Odor, 1952), so that they are unevenly distributed among the blastomeres.

In confirmation of Matthey's (1950) observations, the unusually large sex chromosomes of *M. agrestis* were found to be easily recognizable on the maturation spindles both of spermatocytes and oocytes. Study of the first and second cleavage spindles has now shown the great disparity in size between sex chromosomes and autosomes to be true for these stages. In addition, the *X*- and *Y*-chromosomes can generally be distingusihed from each other. Thus, when suitable chromosome groups are present it is clearly possible to recognize the genetic sex of a vole embryo at a very early stage, even whilst it is still a single cell. In common with the autosomes the

cleavage sex chromosomes are very much larger, particularly on the first cleavage spindle, than they are on the maturation spindles. The X-chromosome on the first meiotic spindle in the oocyte is about $7 \cdot 5\,\mu$ long (Matthey, 1950). On the first cleavage spindle it was found to be about $24\,\mu$ long.

In certain respects the vole egg resembles the hamster egg, details of which have recently been published (Austin, 1956*a*). Dissolution of the zona pellucida occurred with solutions more acid than pH 2·7; the corresponding hydrogen-ion concentration in the hamster was pH 3·0. For comparison, the values for rat and rabbit eggs are pH 5 and 2 respectively (Hall, 1935; Harter, 1948; Braden, 1952). Like the hamster egg the vole egg was always found to have an intact first polar body when seen shortly after ovulation. Persistence of the first polar body is observed in about 1·3 % of rat eggs (Austin & Braden, 1954), 9–54 % of mouse eggs, varying with strain of mouse (Braden, 1956, personal communication), and 88 % of rabbit eggs (unpublished data on 117 eggs). As in most mammalian eggs the second meiotic division proceeds to the metaphase stage before ovulation and normally continues only after spermatozoon penetration.

The vole egg is similar to the hamster egg also in the fact that in neither has a supplementary spermatozoon been found and yet polyspermic specimens of both have been seen. This implies that the zona reaction (Braden, Austin & David, 1954) in the eggs is rapid in its development and constitutes the principal protection against polyspermy. The block to polyspermy in the surface of the vitellus is apparently inoperative. A similar situation is thought to exist in a number of animals, including the dog and sheep. On the other hand, the zona reaction is relatively slow and the block to polyspermy important in the rat and mouse; in the rabbit there is evidence only for the development of a block to polyspermy. The incidence of polyspermy observed in the vole was about 2 %, compared with 1·2 and 1·4 % in the rat and rabbit, respectively (Austin & Braden, 1953), 1·2 % in the mouse (Braden *et al.* 1954), and 1·1 % in the hamster (Austin, 1956*a*). Unlike the hamster egg (Austin, 1956*b*) the vole egg does not seem to possess an array of cortical granules that disappear upon spermatozoon penetration.

In the vole the interval between coitus and the first cleavage of the egg was found to be about 24 hr. This is a little longer than in the rabbit (22 hr., Gregory, 1930), but shorter than in the ferret (54 hr., Hamilton, 1934)—these animals also having coitus-induced ovulation.

Very little evidence of spontaneous activation was seen in vole eggs; indeed, only one unpenetrated egg with a single large nucleus was seen. Chromosome scatter seemed to be the more usual occurrence in unfertilized eggs. In this way the vole egg resembled that of the rat in which spontaneous activation was seen in only 0·9 % of eggs (Austin & Braden, 1954); in the hamster, on the other hand, about 80 % of unpenetrated eggs undergo spontaneous activation (Austin, 1956*c*).

SUMMARY

The spermatozoon has a form that is common to many murine rodents, but the apex of the head is more strongly recurved than usual. The length of the whole spermatozoon is about $104\,\mu$, the head $7\,\mu$ and the mid-piece $27\,\mu$.

The egg is smaller than previously recorded smallest mammalian eggs; the overall diameter is about 96 μ and the diameter of the vitellus during fertilization about 60 μ. The zona is 10 μ thick. No significant degree of contraction of the vitellus was noted after spermatozoon penetration. The zona pellucida dissolved in solutions having a pH of less than 2·7. The first polar body and the metaphase of the second meiosis were seen in all unpenetrated eggs observed shortly after ovulation. From 28 to 360 spermatozoa were found at the site of fertilization (mean 124). The mean number of ovulated eggs per vole was 4·1. In mated animals about 82 % of eggs were fertilized. Mean volumes of larger and smaller pronuclei were 2013 and 1594 μ^3, and corresponding total nucleolar volumes were 116 and 105 μ^3 respectively. The first cleavage of the egg occurred 24 hr. after coitus. Eggs began passing into the uterus between 40 and 50 hr. after coitus, even though some were still in the 4- and 6-cell stages. No supplementary spermatozoa were seen and the incidence of polyspermy was about 2 %. The zona reaction is therefore very rapid and the block to polyspermy probably inoperative. In about 45 % of penetrated eggs both the mid-piece and main-piece of the tail of the fertilizing spermatozoon had failed to enter the vitellus. This has apparently no deleterious effect on the development of the embyro. The sex chromosomes on the early cleavage spindles, particularly the first, are very large and the genetic sex may be recognized even whilst the embryo is still a single cell.

The ultra-violet photomicrograph shown in Pl. 1, fig. 2, was taken by Mr M. Young, at this Institute. The author's thanks are due also to Miss J. Lawrence who prepared the photographic enlargements.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Spermatozoon from the vas deferens. Phase-contrast. ×950.

Fig. 2. Spermatogonial metaphase from a testis-squash preparation. Acetocarmine fixation. Ultra-violet (2750 Å). ×2350.

Fig. 3. Chromosome group in the metaphase of the first meiosis, from a testis-squash preparation. Acetocarmine fixation. Phase contrast. ×950.

Fig. 4. First polar spindle in a follicular oocyte, showing the two *X*-chromosomes. Histological preparation: Bouin fixation, haematoxylin and eosin staining. Phase-contrast. ×950.

Fig. 5. Second polar spindle in an ovulated oocyte, showing characteristic rounded shape with acute convergence at the poles. Fixed under cover-slip with acetic-alcohol, stained with toluidine blue. Phase-contrast. ×950.

Fig. 6. Same egg as in fig. 5, at slightly different focus, showing the large *X*-chromatids. Direct illumination.

Fig. 7. Chromosome group of second polar spindle in an ovulated oocyte, seen in polar view. The *X*-chromatids are clearly distinguishable by their size. Fixed under cover-slip with acetic-alcohol, stained with toluidine blue. Direct illumination. ×950.

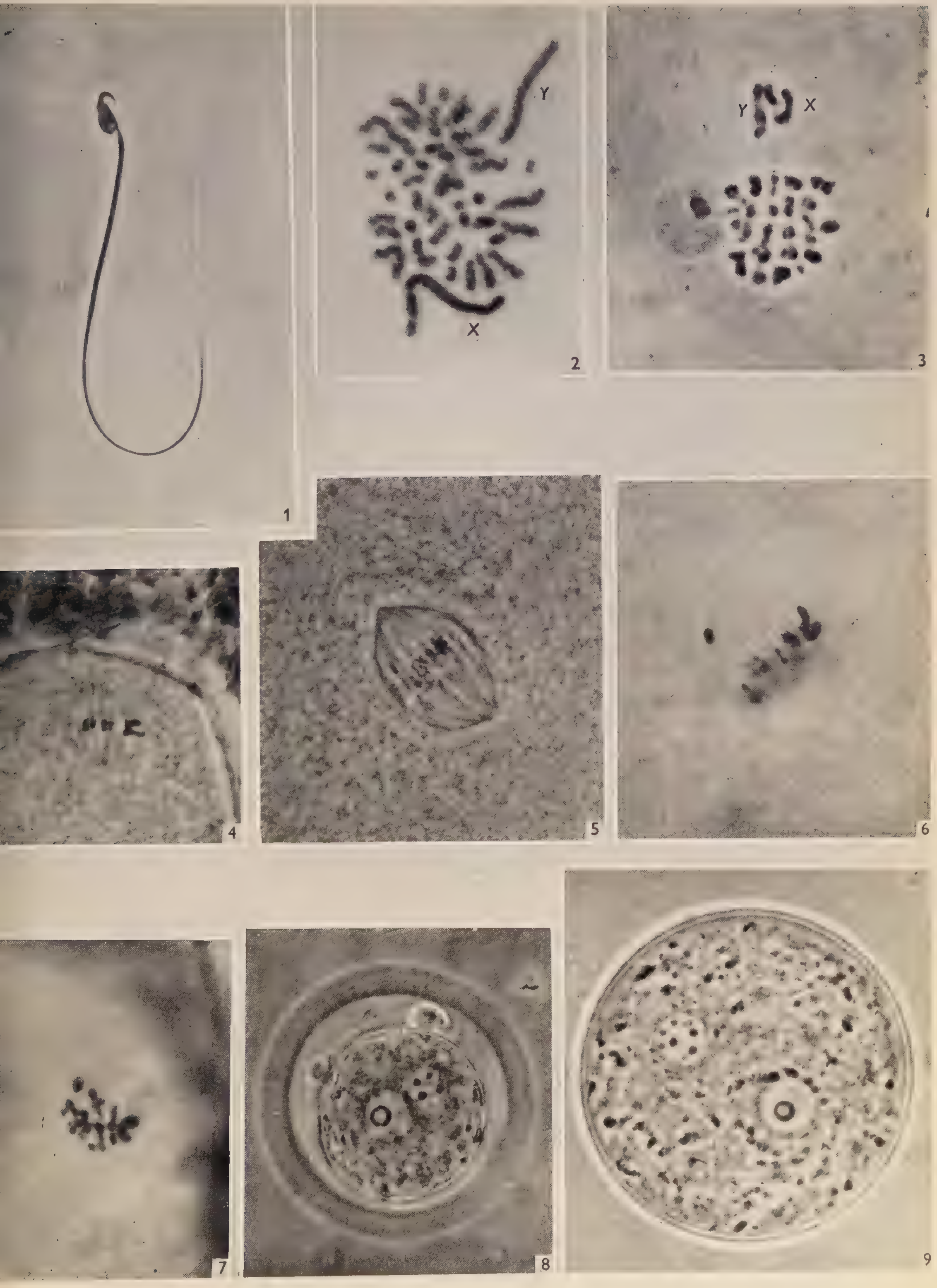
Fig. 8. Egg recovered during course of fertilization, showing two pronuclei and, in the perivitelline space, the mid-piece and main-piece of the fertilizing spermatozoon lying between the two polar bodies. Egg not compressed. Phase-contrast. ×400.

Fig. 9. A similar egg to that shown in fig. 8, but compressed between cover-slip and slide so that the structure of the pronuclei and of the cytoplasm can be more easily seen. Phase-contrast. ×400.

Plate 2

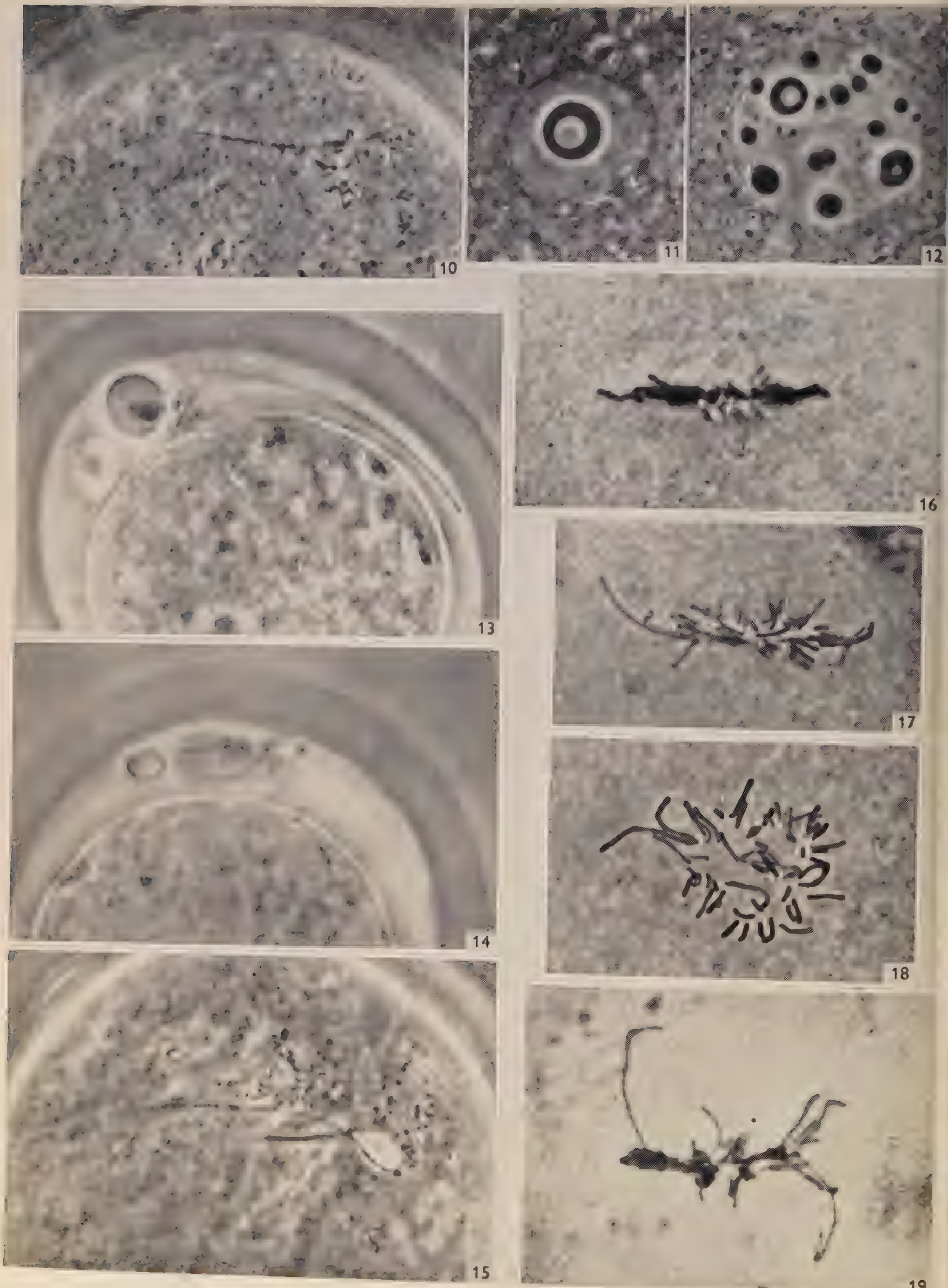
Fig. 10. Spermatozoon mid-piece and main-piece lying within the vitellus. Phase-contrast. ×800.

Fig. 11. Pronucleus in which the nucleolus contains an inclusion within an inclusion. Phase-contrast. ×950.

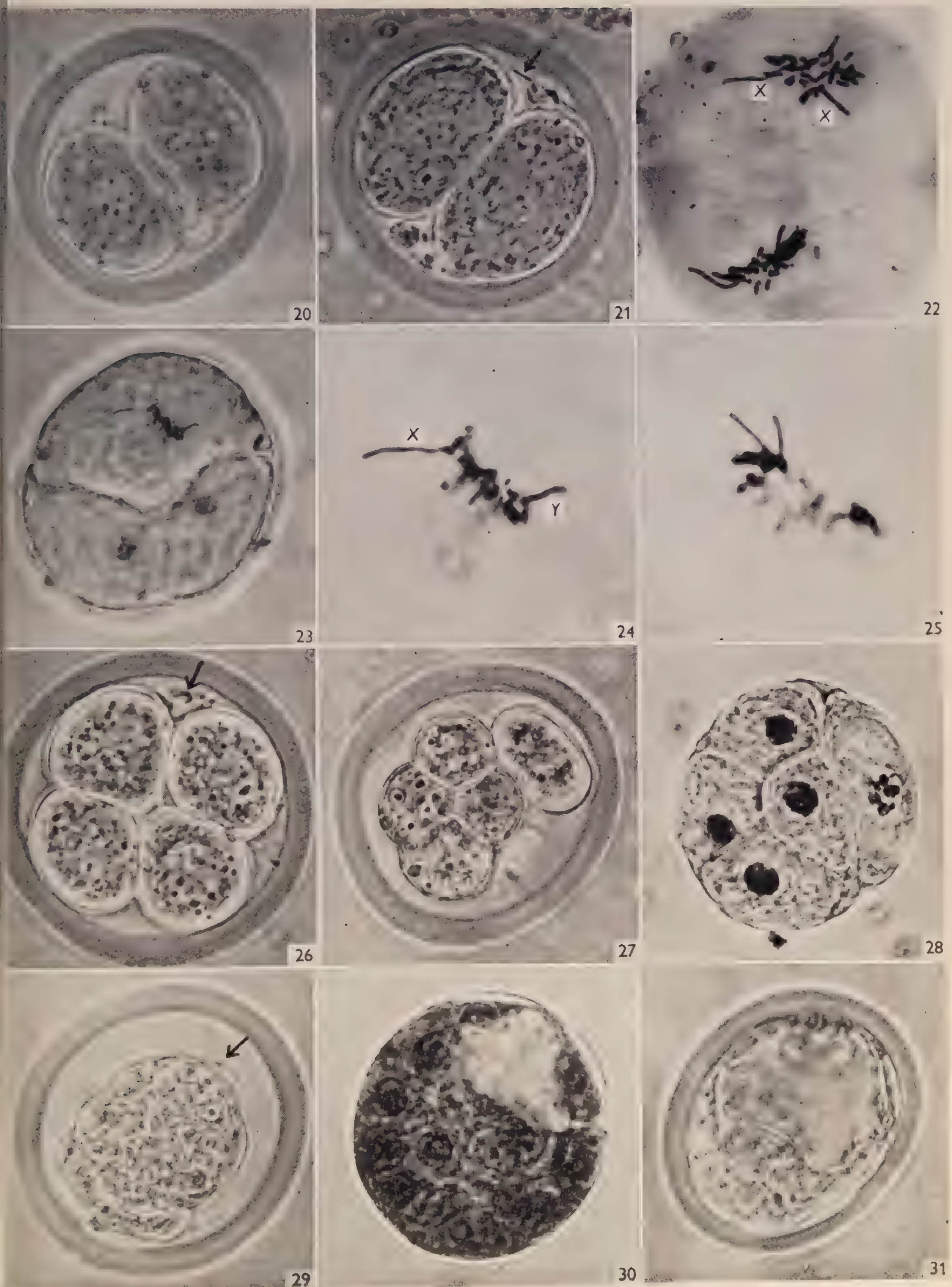


AUSTIN—Fertilization and early cleavage in the vole

(*Facing p.* 10)



AUSTIN—FERTILIZATION AND EARLY CLEAVAGE IN THE VOLE



AUSTIN—Fertilization and early cleavage in the vole

Fig. 12. A single large spontaneously formed nucleus in an unpenetrated egg recovered on the day following ovulation. Phase-contrast. ×950.

Figs. 13–15. These show the presence in the perivitelline space of the tail of the fertilizing spermatozoon. In the 2-cell egg illustrated in Fig. 15 this structure lies on top of the blastomeres, the mid-piece having acquired a somewhat granular appearance. Phase-contrast. Figs. 13 and 14, ×800; Fig. 15, ×1000.

Figs. 16–19. Four eggs exhibiting the first cleavage spindle.

All these eggs were fixed under the cover-slip with acetic-alcohol, stained with toluidine blue and then compressed.

Fig. 16. The wide metaphase plate, extending well beyond the bounds of the spindle; there is a suggestion of centrosomes at the poles of the spindle, particularly at the upper one. Phase-contrast. ×800.

Fig. 17. There appeared to be only one X-chromosome present in this group—it can be seen on the left, and has a close resemblance in form and disposition to the X-chromosome in fig. 18. Phase-contrast. ×950.

Fig. 18. A metaphase group seen in polar view; the X-chromosome is clearly shown on the left and the Y-chromosome could be recognized in the centre of the group though it is not obvious at this focal plane. The genetic constitution is thus male. The X-chromosome was found to be 24μ in length. Phase-contrast. ×950.

Fig. 19. A group showing two X-chromosomes, one on each side. The egg is thus a genetic female. Direct illumination. ×950.

Plate 3

Fig. 20. A 2-cell egg, slightly compressed to show the nuclei. Phase-contrast. ×400.

Fig. 21. A 2-cell egg with the tail of the fertilizing spermatozoon (arrow) in the perivitelline space.

Fig. 22. A 2-cell egg fixed under the cover-slip with acetic-alcohol, stained with toluidine blue, and compressed. Two X-chromosomes were readily identified on the uppermost of the two second cleavage spindles. The embryo is thus a genetic female. Direct illumination. ×400.

Fig. 23. A 3-cell egg fixed under the cover-slip with acetic-alcohol, stained with toluidine blue, and compressed. A second cleavage spindle is evident in the largest blastomere. Phase-contrast. ×400.

Fig. 24. A study at higher magnification of the chromosome group in the egg shown in fig. 23. Probable X- and Y-chromosomes are seen at opposite sides of the metaphase plate (=genetic male). Direct illumination. ×950.

Fig. 25. Chromosome group from a similar egg to that shown in fig. 23. Two large chromosomes are evident that seemed to be of the same kind. It is presumed that two X-chromosomes are present and that the embryo is a genetic female. Direct illumination. ×950.

Fig. 26. A 4-cell egg that was recovered from the uterus approximately 50 hr. after coitus. The egg also shows (arrow) the headless remains of a spermatozoon, apparently reduced in size, in the perivitelline space. Phase-contrast. ×400.

Fig. 27. A 5-cell egg from the Fallopian tube with a large elongated blastomere. Phase-contrast. ×400.

Fig. 28. The same egg as in fig. 27 after it had been fixed with acetic-alcohol, stained with toluidine blue and somewhat compressed. The large blastomere is seen to contain a nucleus in which chromosome condensation is taking place preparatory to the formation of a third cleavage spindle. Phase-contrast. ×400.

Fig. 29. A morula recovered from the uterus about 50 hr. after coitus. In the perivitelline space a structure can be seen (arrow) that is presumed to be the tail of the fertilizing spermatozoon. Phase-contrast. ×400.

Fig. 30. An early blastocyst with a small cavity, recovered from the Fallopian tube about 70 hr. after coitus. Fixed under the cover-slip with acetic-alcohol, stained with toluidine blue and somewhat compressed. Phase-contrast. ×400.

Fig. 31. A later blastocyst recovered from the uterus about 70 hr. after coitus. Phase-contrast. ×400.

OBSERVATIONS ON THE SO-CALLED THYMUS II OF BIRDS

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INTRODUCTION

The epithelial anlage of the definitive thymus of birds is usually thought to develop from the endoderm of the IIIrd pharyngeal pouch, with a small contribution from the IVth pouch. In the emperor penguin embryo, Glenister (1954) has described, in addition to thymus III and IV, a thickening of the epithelium of the dorsal part of the IInd pouch, and a similar thickening had been noted previously by Kastschenko (1887) in the chick embryo. In both chick and emperor penguin the thickening disappears without undergoing any specific differentiation, but both authors regard it as an abortive thymus, 'representing' the thymus II of lower vertebrates. This so-called thymus II is larger and longer-lived in the emperor penguin than in the chick, and Glenister (1954) advances this fact in support of his general conclusion that the emperor penguin is a very primitive bird.

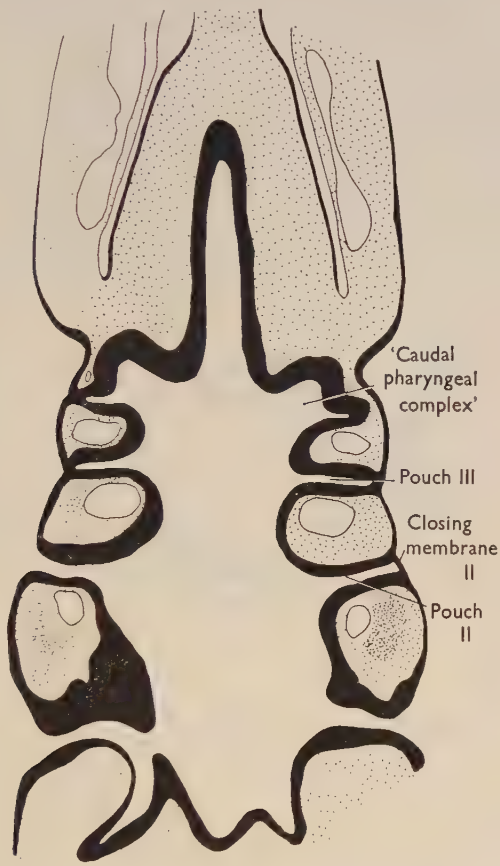
The present investigation has two main aims: to find out if thymus II is of general occurrence in birds and to determine whether it arises from the pharyngeal endoderm or from the branchial ectoderm. The significance of the second aim is that Hammond (1954) has recently questioned the generally held view that thymus III and thymus IV in the chick are endodermal derivatives. He concludes from a study of normal and experimental embryos that the thymus is a derivative of the branchial ectoderm, which he describes as becoming secondarily incorporated within the caudal wall of the dorsal parts of pouches III and IV. It is of interest therefore to know if the so-called thymus II is also of ectodermal origin.

MATERIAL

Embryos of gannet, black-headed gull, moorhen, pigeon, thrush and blackbird have been studied. Although the selection of these particular species for study was largely fortuitous, being determined by availability, it does in fact form a fairly representative cross-section of the avian orders, ranging from the gannet, a member of the relatively primitive order, Pelicaniformes, to the Passerines, the thrush and blackbird. Most of the embryos were fixed in Bouin's fluid, embedded in paraffin, sectioned at $8\,\mu$ and stained with haematoxylin and eosin. Two of the pigeon embryos were fixed in an alcohol-formalin-picric-acid mixture, and stained for glycogen by the periodic acid-Schiff method. The embryos were measured after fixation; the length given in each case is the greatest length. In some cases the approximate stage of development is indicated by comparison with the developmental stages of the chick (Hamburger & Hamilton, 1951).

OBSERVATIONS

For convenience the epithelial thickening will be referred to as thymus II, without prejudice to the later discussion of its nature and significance.



Text-fig. 1. Gannet, 7 mm. Horizontal section through pharynx to show general relations of pouches. ×67.

Gannet (Sula bassana) (7, 9, 11, 13, 14, 16·5 *and* 28 *mm.*)

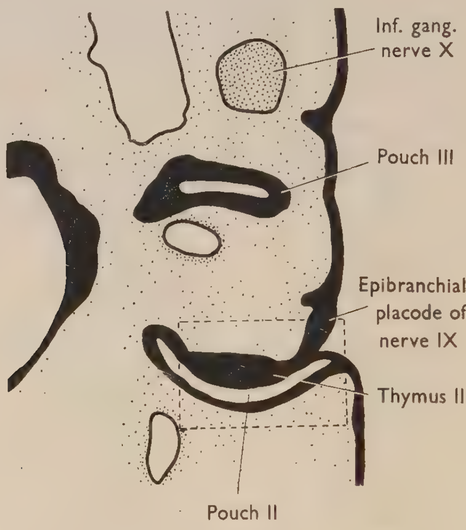
In the gannet, thymus II is relatively large and long-lived. Its origin can be most clearly appreciated in sections passing horizontally through the pharynx, i.e. in a plane at right angles to the long axis of the IInd arch. Text-fig. 1 is from a 7 mm. embryo sectioned in this plane. It shows the 1st, 2nd and 3rd pouches and the 'caudal pharyngeal complex'. Internally, the pouches are widely continuous with the general pharyngeal cavity, externally each (on one side, or both) makes contact with the ectoderm at a closing membrane.

The IInd closing membrane is intact throughout its extent on the left side of this embryo, but is ruptured on the right side except at its dorsal (or upper) and ventral

(or lower) ends. Text-fig. 1 shows that the membrane makes a small angle with the parasagittal plane. It consists of three or four layers of cells, and it is not possible to distinguish those of ectodermal from those of endodermal origin.

Thymus II is not yet clearly recognizable in the 7 mm. embryo, but Pl. 1, fig. 1, shows the region in which it will later develop. The section passes through the dorsal end of the pouch, some 140 μ dorsal to that illustrated in Text-fig. 1. The caudal wall of the pouch is thicker than the rostral wall, but the thickened area is not sharply demarcated from the remainder of the caudal wall.

The thickened ectoderm overlying the IIIrd arch immediately caudal to the IInd closing membrane is the ventral end of the epibranchial placode of the IXth nerve.



Text-fig. 2. Gannet, 9 mm. Shows thickening of endoderm of caudal wall of pouch II. $\times 67$.

9 mm. embryo (Text-fig. 2)

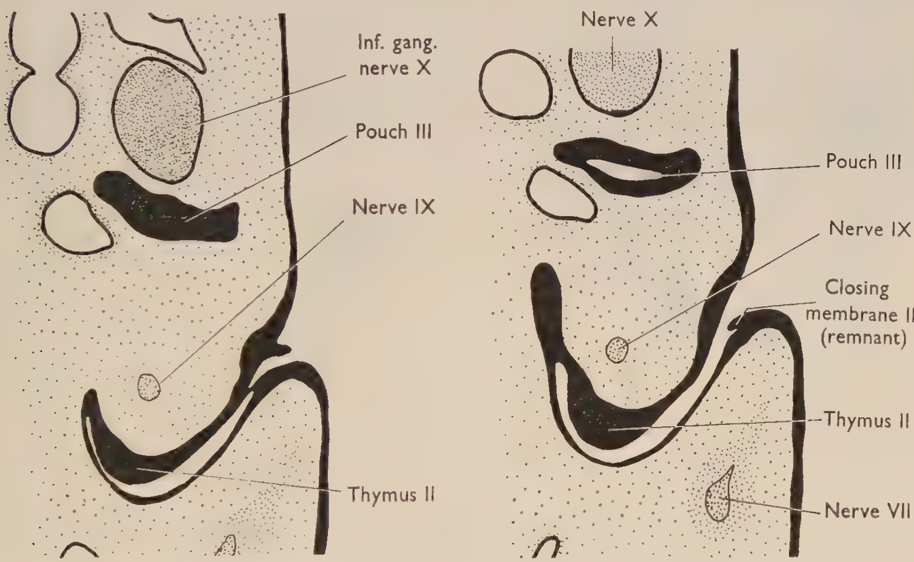
The thickening of the caudal wall of the pouch is now more obvious and more clearly delimited. It lies entirely internal to the closing membrane and is undoubtedly endodermal. The rostral and caudal walls of the pouch have both become convex forwards, and the closing membrane has rotated from a parasagittal into the coronal plane. The altered curvature of the pouch and the rotation of the closing membrane are probably both due to the overgrowth of the IIIrd arch by the IInd arch.

The thickening extends dorsally to within about 100 μ of the ventral end of the inferior ganglion of the IXth nerve. Its medial and lateral limits are fairly clearly defined, but dorsally and ventrally it fades away gradually into the general pouch wall. Its total dorso-ventral extent is approximately 110 μ. At its centre, there are some thirteen to fourteen layers of nuclei, as compared with about four layers in the unthickened rostral wall (Pl. 1, fig. 2). Cell boundaries are not evident and with the H & E stain there are no tinctorial differences between the thickened area and the remainder of the pouch.

It is not clear what produces the thickening of the caudal wall of the pouch. Mitoses are no more numerous there than elsewhere in the pouch, and the thickening seems far greater than could be accounted for by compression of the more acutely curved caudal wall. It may be that the thickening comes into existence as the result of morphogenetic movements such as lead to the appearance of the epithelial anlagen of the hairs and mammary gland (cf. Balinsky, 1950).

10 *mm. embryo*

The overgrowth of arch III by arch II has continued. Pouch II is more markedly convex rostrally, and the rotation of the closing membrane has continued so that its external surface, which at 7 mm. faced almost directly laterally, now faces caudomedially (Text-fig. 3). Outside the closing membrane, the ectoderm of the IIIrd arch has fused with that of the overlapping IInd arch. In the section illustrated in Text-fig. 3 an artifactual split extends into the area of fusion. The thickening of the



Text-fig. 3 Text-fig. 4

Text-figs. 3, 4. Gannet, 10 mm. ×67.

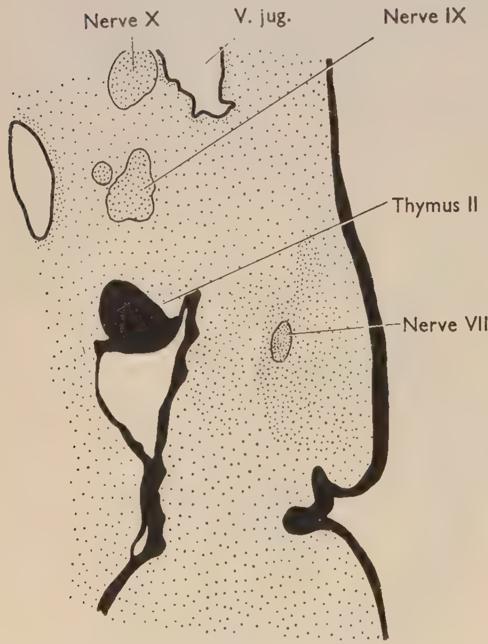
caudal wall of the pouch is evident. Text-fig. 4 shows a more ventral level of section, passing through the thickest part of the swelling. Although the closing membrane is ruptured at this level, comparison with more dorsal levels of section, where the membrane is still intact, confirms that the thickening consists of pharyngeal endoderm, without any ectodermal contribution.

The dorsoventral extent of the thickening is about 125 μ. As in the 9 mm. stage, mitoses are no more numerous in the thick than in the thin portion of the wall, and the thickened area has no cytological peculiarity.

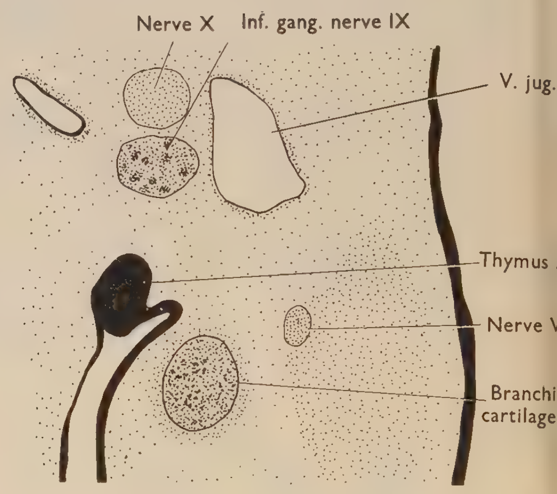
Caudal to thymus II, and in the same parasagittal plane, lies the IXth nerve. This relationship persists in the later stages of development, and is of some value in comparing conditions in the gannet with those in other birds.

11 mm. embryo

The dorsal one-half or more of pouch II is now widely separated from the surface ectoderm. Thymus II reaches its greatest size, and extends dorsoventrally for about $250\,\mu$. It is no longer an entirely intramural thickening, but bulges caudally like a sessile polyp. Caudal to it is the IXth nerve (Text-fig. 5) and its inferior ganglion. These relations are illustrated clearly by Pl. 1, fig. 3, a low-power view of a parasagittal section.



Text-fig. 5. Gannet, 11 mm. ×67.



Text-fig. 6. Gannet, 13 mm. ×67.

Mitoses are slightly more numerous in thymus II than at the 10 mm. stage, and the nuclei are somewhat more loosely arranged. All the cells appear quite healthy (Pl. 1, fig. 4).

13 mm. embryo

Thymus II is slightly smaller in this embryo, and measures $200\,\mu$ in dorsoventral extent. Its general form and relations are unchanged (Text-fig. 6), but Pl. 2, fig. 5, illustrates two histological changes which seem to be degenerative: the appearance within the epithelium of small clear spaces ('vacuoles'), and of darkly stained spherical bodies of varying size, which probably represent nuclear debris.

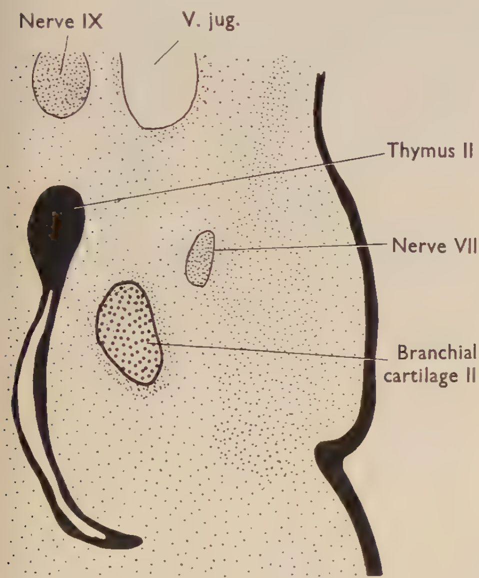
14 mm. embryo

Thymus II is further reduced in size and now measures about $150\,\mu$ in dorsoventral extent. It is pedunculated (Text-fig. 7), and although it shows the degenerative signs noted at 13 mm., most of its cells appear healthy, and mitoses can be found.

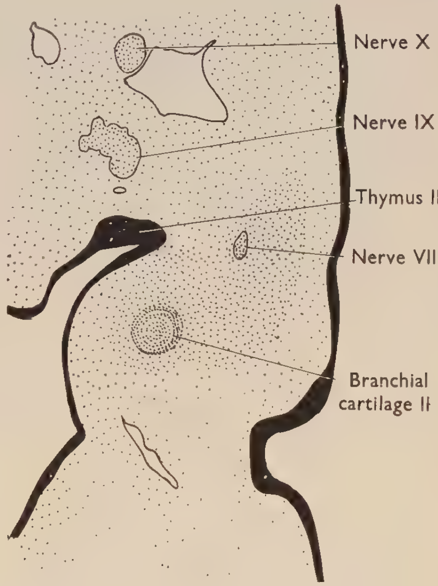
In this embryo parathyroids III and IV may be differentiated histologically from thymus III and IV, a distinction which is not possible earlier. Neither thymus III nor thymus IV shows the degenerative changes which are present in thymus II.

16·5 *mm. embryo*

Thymus II has now become inverted and projects into the lumen of the pouch (Pl. 2, fig. 6). It is more basophilic because of nuclear pyknosis, and looks degenerate, with many irregular vacuoles and much nuclear debris. It is obviously regressing. Exactly when it disappears has not been determined, but no trace of it can be found in the next older embryo studied, one of 28 mm.



Text-fig. 7. Gannet, 14 mm. ×67.



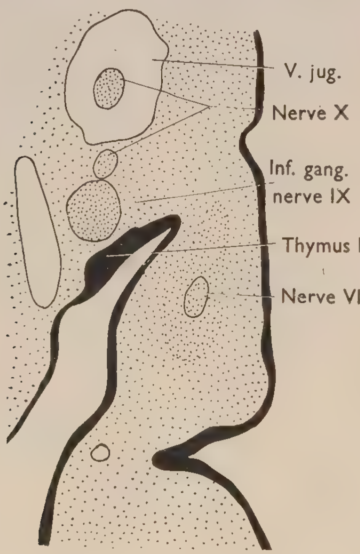
Text-fig. 8. Black-headed gull, 9·5 mm. ×67.

Black-headed gull (Larus ridibundus) (5, 7, 8, 9·5, 14, *and* 25 *mm.*)

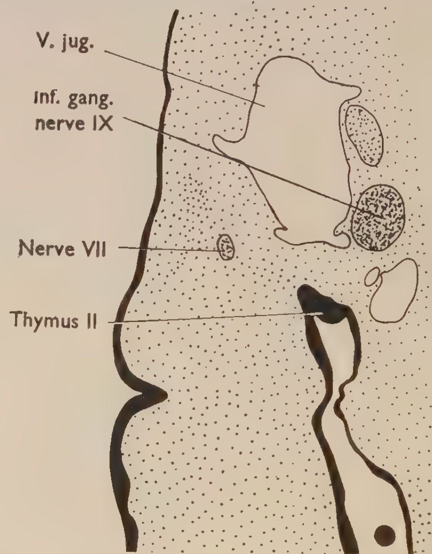
Thymus II is first found in the 9·5 mm. embryo. Its position and relations are illustrated in Text-fig. 8, and comparison with Text-fig. 5 will make it clear that we are dealing with a structure very similar to that already described in the gannet. It forms an intramural thickening (216 μ dorsoventrally) lying directly rostral to the IXth nerve. The cells of the epithelial swelling look healthy (Pl. 2, fig. 7). At 10·5 mm. one or two small areas of vacuolation and nuclear fragmentation are seen, and in the 14 mm. embryo thymus II looks very degenerate and projects like a polyp into the lumen of the pharynx (Pl. 2, fig. 8). Vacuolation and nuclear fragmentation are well marked. No trace of thymus II could be found in the next larger embryo studied, one of 25 mm.

The similarity between thymus II in gannet and gull is obvious, but one cannot be certain that in the gull it is entirely endodermal in origin, as one can in the gannet. The reason for this uncertainty is that by the time thymus II appears, in the 9·5 mm. embryo, the dorsal end of pouch II is already widely separated from the

surface ectoderm. At 5 mm. (Pl. 3, fig. 9) the closing membrane of the IInd pouch is still intact, but it has already rotated into the coronal plane by the backward growth of arch II. At 7 mm. (Pl. 3, fig. 10) continued backward growth of the IInd arch has been responsible (partly at any rate) for the rupture of the closing membrane, which is now represented by two epithelial tags, *a* and *b*. The epithelial tag, *a*, which projects forwards from the caudal wall of the pouch marks the boundary between ectoderm and endoderm. At 8 mm. (Pl. 3, fig. 11) this landmark has disappeared, and, since thymus II has not yet developed, it is clearly impossible to be certain that, when it does appear, it arises solely from pouch endoderm.



Text-fig. 9. Moorhen, 9 mm. ×67.



Text-fig. 10. Pigeon, 9 mm. ×67.

Moorhen (Gallinula chloropus chloropus *L.*) (9, 12, 14 *and* 18 *mm.*)

Thymus II is found in the 9 mm. embryo as an entirely intramural thickening of the caudal wall of pouch II, lying, as in the gannet and the gull immediately rostral to the IXth nerve (Text-fig. 9). It is about 180 μ in dorsoventral extent. At 12 mm. it is slightly less extensive (dorsoventrally, 144 μ). It bulges backwards from the caudal wall of the pouch and shows the familiar signs of incipient degeneration (Pl. 3, fig. 12). At 14 mm. it is almost completely degenerate, and no trace of it can be found in the 18 mm. embryo.

For the same reasons as in the gull, the precise origin of thymus II in the moorhen must remain in some doubt.

Pigeon (Domestic). 8 *mm.* (*stage* 25), 9 *mm.* (*stage* 27), 11 *mm.* (*stage* 28), 12 *mm. and* 20 *mm.* (*stage* 35)

Thymus II is already well developed in the 8 mm. embryo, the youngest examined (Pl. 3, fig. 13). At this stage, the dorsal end of pouch II is no longer in contact with the surface ectoderm and one cannot tell therefore whether thymus II is endodermal or ectodermal in origin. It measures some 200 μ in dorsoventral extent.

Fig. 10 shows the general relations of thymus II in a 9 mm. embryo. Its dorsoventral extent is about 210 μ. It already shows some signs of degeneration.

At 11 mm., the swelling is somewhat smaller and looks very degenerate. However, in a somewhat older embryo of 12 mm. thymus II measures about 230 μ dorsoventrally and its cells look essentially healthy. No trace of the swelling was found in the 20 mm. embryo.

Text-fig. 11. Thrush, 8 mm. ×67.

The 8 and 9 mm. embryos were specially stained for the demonstration of glycogen. In an earlier study on the sheep embryo (Scothorne, 1955), it was shown that, from the time of their earliest differentiation, thymus III and parathyroid III are clearly distinguishable from one another by the fact that the cells of the epithelial thymic anlage contain moderate amounts of glycogen, whereas those of the parathyroid contain none. It was thought that some such difference in glycogen content of thymus and parathyroid might exist in the pigeon. In fact, however, glycogen is absent in thymus II, thymus III and parathyroid III in both the 8 and 9 mm. pigeon embryos, and the method does not, therefore, throw any more light on the nature of the so-called thymus II.

Thrush (Turdus ericetorum ericetorum *Turton.*) (8 *mm., stages* 26–27) *and blackbird* (Turdus merula merula *L.*) (11 *mm., stage* 28, *and* 13 *mm., stage* 30)

In the 8 mm. thrush embryo, a typical thymus II is present. It projects from the caudal wall of pouch II (Text-fig. 11), measures about 200 μ in dorsoventral extent and shows vacuoles and nuclear debris, already noted as signs of degeneration in the other species.

Similar appearances are seen in the 11 mm. blackbird embryo (Pl. 3, fig. 14), but no trace of thymus II is to be found in the 13 mm. embryo.

DISCUSSION

In each of the six species studied, a thickening of the epithelium of the dorsal part of pouch II has been found. A similar thickening was reported previously in the chick (Kastschenko, 1887, Lillie, 1952), duck (Hamilton, 1913) and the emperor penguin (Glenister, 1954). There is no mention of this 2nd pouch thickening in the sparrow (Helgesson, 1913), pied-billed grebe (Johnson, 1918) or the 'Kiebitz' (probably our lapwing) (Sicher, 1921). Unless specifically sought, however, it is easily overlooked, and it seems likely, in view of the present study, that it is of general occurrence in birds.

Table 1. *The occurrence of thymus II in vertebrates other than birds*

Class	Abortive or definitive	Endodermal or ectodermal	Authority
Elasmobranchii	Definitive	Endodermal (partly ectodermal?)	Beard (1903); Antipa (1892); Froriep (1891)
Actinopterygii	Definitive	Partly ectodermal	Deanesly (1928)
Amphibia			
Urodeles	Abortive	Endodermal	Maurer (1888); Baldwin (1918)
Anurans	Definitive	Endodermal	
Reptilia			
Anguis	Definitive	Endodermal	St Remy & Prenant (1904); van Bemmelen (1893); Shaner (1921)
Lacerta	Definitive	Endodermal	
Ophidia	Abortive	Endodermal	
Chelonia	Abortive	Endodermal	
Mammalia			
Marsupials			
Trichosurus	Definitive	Ectodermal and IInd pouch endoderm	Fraser & Hill (1916)
Phascolomys	Definitive	Ectodermal and IInd pouch endoderm	Fraser (1916)
Eutheria			
Lepus	Abortive	Endodermal	Piersol (1888)

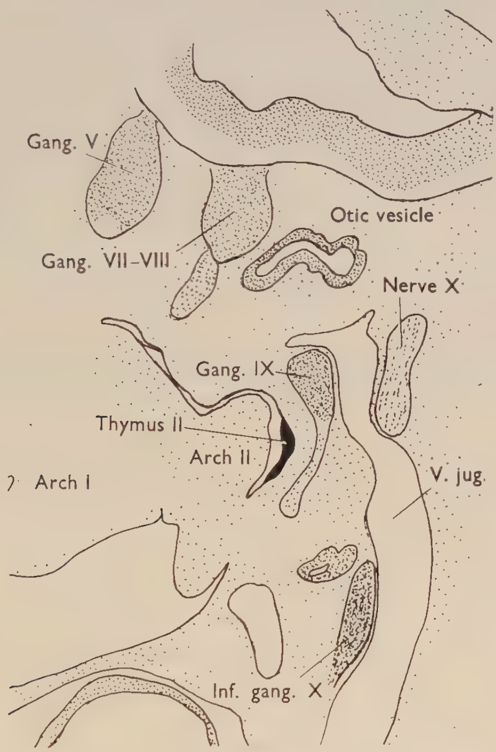
Since the thickening is ephemeral and undergoes no specific histological differentiation, one can only speculate about its nature. The evidence of comparative embryology is consistent with the view that it is an abortive thymus. Table 1 summarizes the occurrence of thymus II in vertebrates other than birds; the presence of a definitive thymus II in some reptiles is of particular interest in view of the close phylogenetic relationship between birds and reptiles (cf. Howard, 1950).

The position of the thickening in birds is also consistent with the view that it is an abortive thymus II. In the mammals, the definitive thymus (in the majority of species, thymus III) develops from the ventral end of the pouch, the dorsal end giving rise to the parathyroid III. In submammalian forms, on the other hand, this relationship is reversed and the thymus arises from, or in relation to, the *dorsal* end of one or more pouches. A thymus II would therefore be expected to develop in relation to the dorsal end of the IInd pouch.

Until recently it has been generally, if not universally, held that the epithelial thymic anlage in birds is of endodermal origin, arising principally from the IIIrd pharyngeal pouch. The experimental studies of Hammond (1954), however, indicate that the epithelium of the thymus is in fact derived from placodal ectoderm which

becomes intimately associated with pouches III and IV. Excision of the epibranchial placodes in 19–27 somite chick embryos results in absence of the thymic cord on the operated side.

These findings have an interesting bearing on the present investigation. If the definitive thymus—thymus III and IV—of the chick (and presumably of other birds as well) is of ectodermal origin, one might expect a similar origin of thymus II.



Text-fig. 12. *Pygoscelis taeniatus*, 9 mm. ×67. Parasagittal section.

But the so-called 'thymus II' has been shown quite definitely to be a pouch (endodermal) derivative in the gannet. The contradiction is, however, only apparent, and invalidates neither the findings of Hammond (1954) nor the thymic nature of the IInd pouch thickening. Comparative studies have long since established that the thymus shows scant respect for the germ layer theory—in the mole, for example, there is found an abortive thymus III derived from the endoderm of the third pouch, while the definitive thymus is ectodermal (Rabl, 1909).

The available evidence is, then, consistent with, but clearly does not establish, the idea that the epithelial swelling associated with the IInd pouch in birds does 'represent' an abortive thymus; thymus II is found in the group most nearly related to the birds—the reptiles—and the epithelial thickening is related to the dorsal part of the pouch where one would expect to find it.

On the available evidence one can go no further than this. Glenister (1954) makes the interesting suggestion that the large and long-lived thymus II in the

emperor penguin is evidence of the primitive character of that bird. From a study of Glenister's photograph and description, thymus II in the emperor penguin is, however, little, if any, larger, and no longer-lived, than that of the gannet. Furthermore, thymus II is not conspicuously large in another species of penguin, *Pygoscelis taeniatus* (Text-fig. 12). Finally a thymus II has been found in every species in which it has been sought in the present study. These species may be arranged in order from more to less primitive: gannet, moorhen, gull, pigeon, thrush and blackbird. Thymus II is undoubtedly best developed, among these species, in the gannet, but the fact that a well-developed thymus II is present in the least primitive species—the thrush and blackbird—suggests that it is probably a character common to the birds as a class and that its relative degree of development is of rather limited value in assessing the phylogenetic position of any particular species within the class.

SUMMARY

1. In the embryos of each of six species of bird there develops a thickening of the epithelium of the caudal wall of the dorsal part of the 2nd pharyngeal pouch.

2. This epithelial thickening is of endodermal (pouch) origin in the gannet. In the other species the possibility of a contribution from the branchial ectoderm cannot be excluded.

3. The thickening is transient and undergoes no specific histodifferentiation before it degenerates, apparently without trace.

4. The available evidence is consistent with the view that the thickening is the abortive anlage of a thymus II.

I am grateful to Dr J. Gibson and to Mr H. Sutherland for their invaluable help in the collection of material; to Mr A. Campbell and Miss C. McCaffery for technical assistance; to Prof. G. M. Wyburn for criticism of the manuscript; and to Mrs C. W. Parsons for her generous loan of the embryonic penguin material studied by the late C. W. Parsons. M.A.

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EXPLANATION OF PLATES

Unless stated otherwise, all photomicrographs are of sections cut horizontally through pharynx. For general orientation, compare with Text-fig. 1.

Plate 1

Fig. 1. Gannet, 7 mm. ×117.

Fig. 2. Gannet, 9 mm. Higher power photomicrograph of rectangular area marked out in Text-fig. 2. ×270.

Fig. 3. Gannet, 11 mm. Parasagittal section to illustrate position and relations of thymus II. ×34.

Fig. 4. Gannet, 11 mm. ×270.

Plate 2

Fig. 5. Gannet, 13 mm. Note clear spaces ('vacuoles') within, or among, the cells and the small, darkly stained spheres, which probably represent nuclear debris. ×270.

Fig. 6. Gannet, 16·5 mm. Thymus II now looks darkly stained and 'degenerate'. ×270.

Fig. 7. Black-headed gull, 9·5 mm. Cells of thymus II appear healthy. ×270.

Fig. 8. Black-headed gull, 14 mm. Thymus II shows degenerative changes similar to those seen in the gannet. (Cf. fig. 6.) ×270.

PLATE 3

Fig. 9. Black-headed gull, 5 mm. Closing membrane of pouch II is intact and lies in coronal plane. $\times 117$.

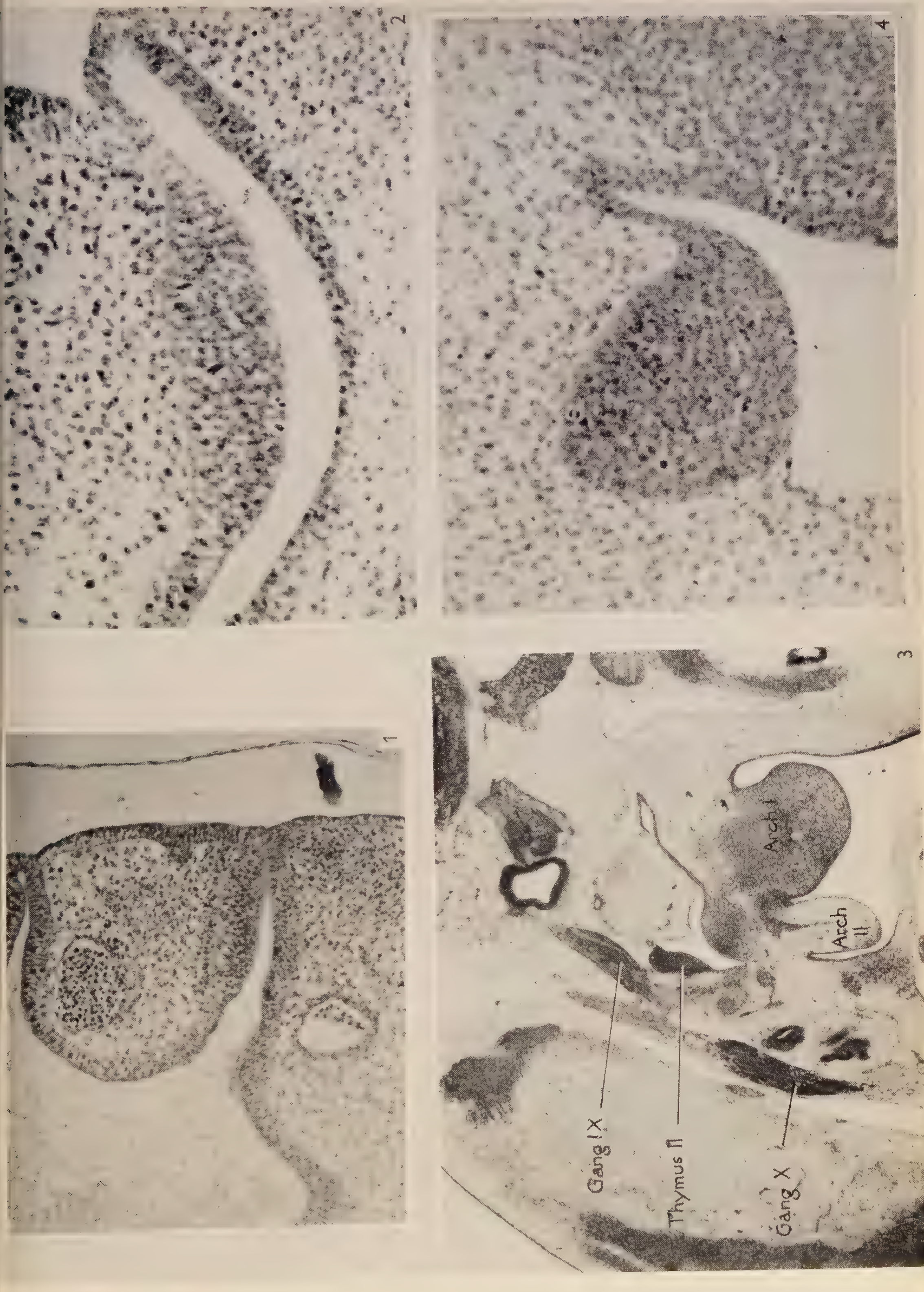
Fig. 10. Black-headed gull, 7 mm. Closing membrane of pouch II has ruptured, and is represented by the two epithelial tags *a* and *b*. The epithelial tag *a* marks the boundary between pouch endoderm and branchial ectoderm. $\times 117$.

Fig. 11. Black-headed gull, 8 mm. All traces of the closing membrane have now disappeared. Comparison of figs. 9–11 will show how the 'backward' growth of arch II moulds the form of pouch II. $\times 117$.

Fig. 12. Moorhen, 9 mm. $\times 270$.

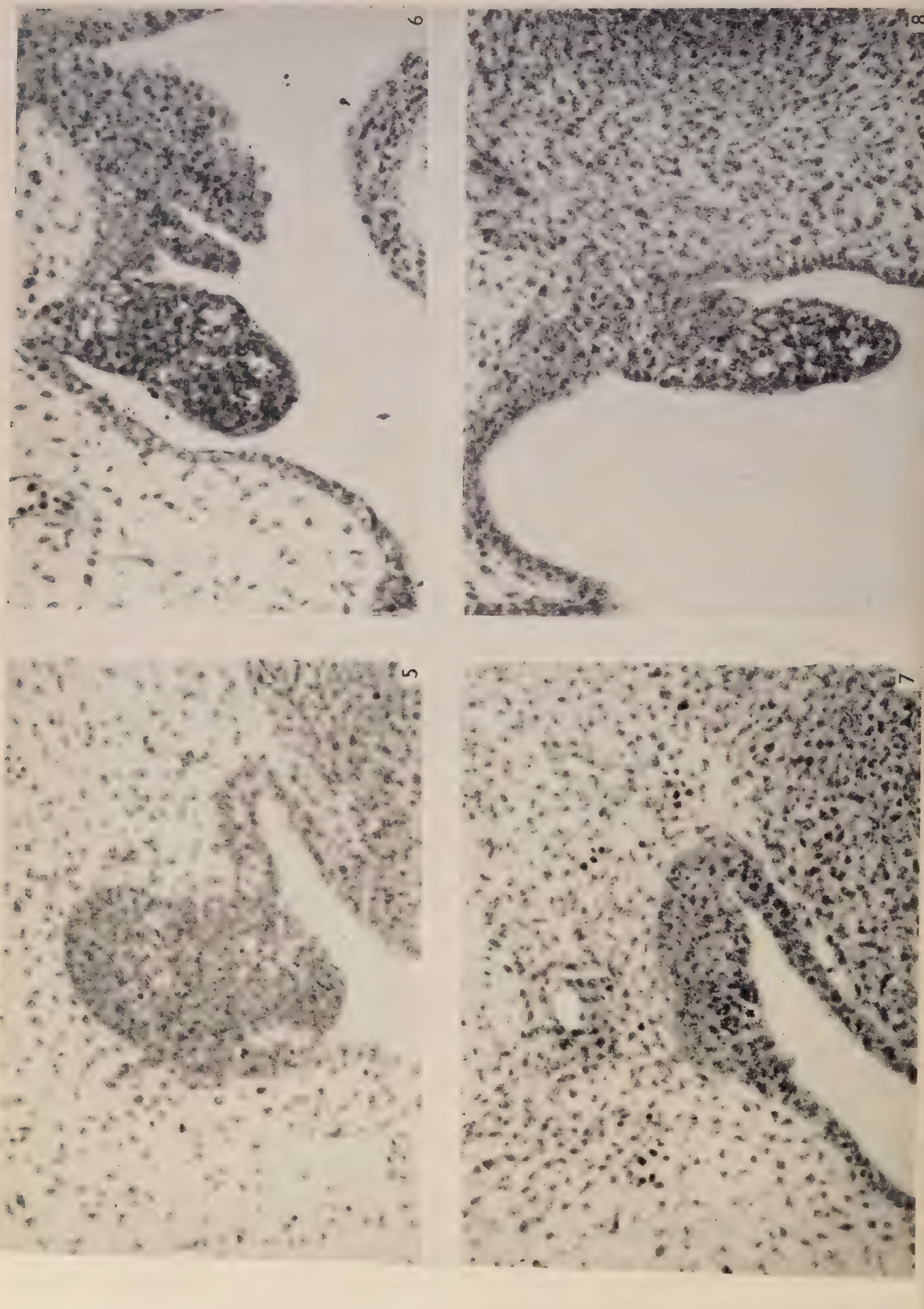
Fig. 13. Pigeon, 8 mm. $\times 270$.

Fig. 14. Blackbird, 11 mm. $\times 270$.



SCOTHORNE—OBSERVATIONS ON THE SO-CALLED THYMUS II OF BIRDS

(*Facing p* 24)



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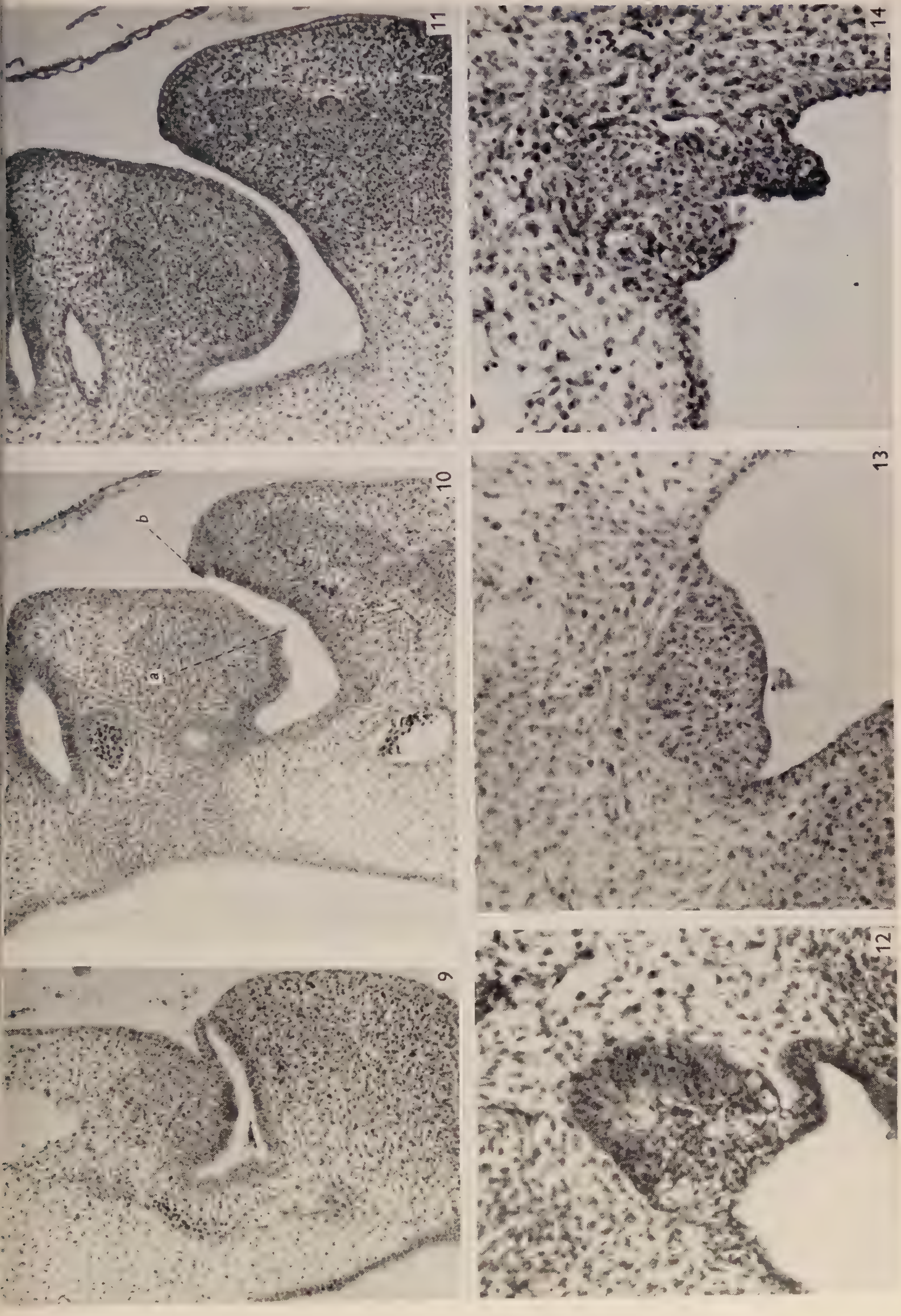

SCOTHORNE—OBSERVATIONS ON THE SO-CALLED THYMUS II OF BIRDS

THE DEVELOPMENT OF THE HIND-BRAIN ARTERIES IN THE RAT

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During the investigation of a case of an anomalous basilar artery in a human subject (Morris & Moffat, in the press), it became apparent from a search of the literature that there are comparatively few detailed accounts of the development of the arteries of the hind-brain and cranial end of the spinal cord. In particular it was noticed that surprisingly few authors have mentioned a prominent longitudinal vessel which runs cranially, dorsal to the rootlets of the hypoglossal nerve and which plays an important part in the blood supply of the hind-brain at one stage of its development.

The only detailed studies of the later stages of development of the arteries of the hind-brain in mammals have been those of Schmeidel (1932) and Padget (1948), who studied human embryos, and of Tandler (1902), who described both rat and human embryos. Apart from these papers there are very many references to the subject scattered throughout the literature, dealing with conditions both in man and in various animals. Since many of these references occur in descriptions of single embryos, it is not surprising that one meets with a number of conflicting accounts. The vessels of the hind-brain region are subject to considerable variation, both in the adult and in the embryo, and it was thought that a study of a comparatively large number of embryos would be necessary to obtain a reasonably accurate picture. For this reason it was decided to investigate the hind-brain arteries of rat embryos by the injection technique. The choice of animal proved fortunate, since in the rat the longitudinal vessel on the side of the hind-brain which was mentioned above persists into adult life to form a branch of the vertebral artery.

MATERIAL AND METHODS

It was first necessary to study the pattern of vessels in the adult rat, since there does not appear to be any satisfactory account of this in the literature. Twenty-six albino or hooded rats were injected with Neoprene latex 572 through the aorta, and after the heads had been fixed in 10% formol saline the vessels of the brain were dissected; a number of newborn rats were studied by the same method. This part of the work was done in conjunction with Dr E. D. Morris.

For the embryological studies, the onset of oestrus was determined by the examination of daily vaginal smears, and at the appropriate time male rats were introduced into the cage for periods of from 3 to 6 hr. Further smears were then taken to ascertain whether copulation had occurred. Successfully inseminated rats were killed at known intervals after mating, and the embryos injected with Higgins's indian ink diluted with an equal quantity of distilled water. It was found that the simplest techniques gave extremely good results and so the following method was used.

Very fine glass cannulae are prepared and connected to a mouthpiece by means of a short length of rubber tubing. A small amount of indian ink is drawn into the cannula, followed by sufficient normal saline to fill the tip. The use of mouth pressure has the advantage that both hands are free to manipulate the specimen and that the pressure can easily be controlled. After opening the pericardium, the tip of the needle is placed against the ventricular wall and is made to enter the cavity of the heart by means of a sharp stab. A small amount of ink is then injected by blowing gently and if the heart is not beating already the presence of the ink usually causes contraction to commence, after which it is only necessary to keep the heart full of ink by injecting a small quantity during each diastolic interval.

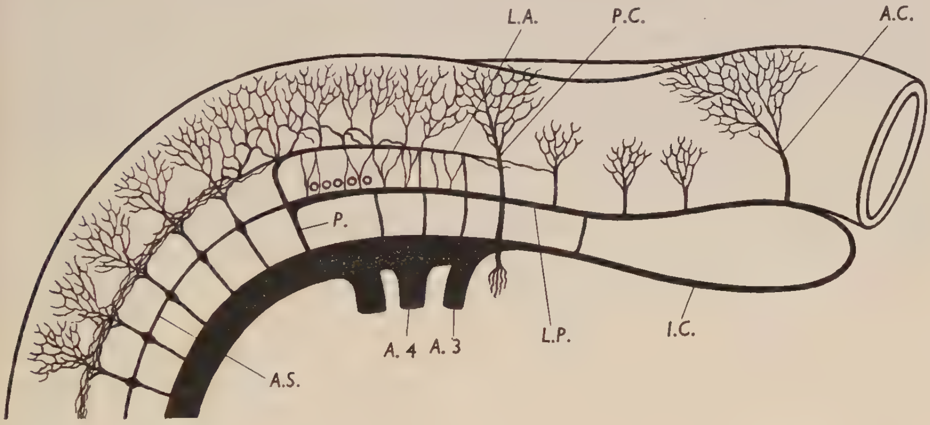
If the injection is to be successful, the arterial tree fills rapidly and almost completely with the first ten or twelve contractions of the heart. Sometimes, however, for no apparent reason, the larger vessels fill at once but the ink hardly passes into the smaller arteries at all. Increasing the pressure of the injection is worse than useless, since it causes the ink to blow back suddenly into the venous system, which increases the difficulty of the subsequent dissection. It must be emphasized that the injection of the arteries is carried out by the contraction of the heart itself and not by forcing the ink into the embryo, except that in the final stages of the injection a little extra pressure may sometimes complete the filling of a vessel which is poorly injected. A number of embryos were injected through the dorsal aorta and in these the same principle holds good. The pressure of the injection causes the ink to flow cranially to the heart, but none of the branches of the aorta is well filled until this ink has been expelled from the heart into the vascular tree. With larger embryos it is possible to inject one of the tributaries of the umbilical vein, the embryo remaining untouched inside the intact amniotic cavity. This method gives the most perfect injection of the two sides since if the injection is made after removing the membranes, the side of the embryo in contact with the slide is often poorly filled.

After injection the embryos are fixed in 10% formol saline and dissected under a binocular microscope, using watchmaker's forceps and fine glass needles and hooks. Fifty-two embryos were studied, of crown-rump lengths varying from 4 to 10 mm.

RESULTS

The pattern of vessels in the adult rat is subject to considerable variation, and the following is only a condensed account of our main findings, and can be followed by reference to Pl. 2, fig. 6, in which the vessels have almost reached their adult arrangement. There are two arteries which take part in the blood supply of the cerebellum. The anterior cerebellar artery arises from the terminal part of the basilar artery, runs dorsally and supplies branches to the greater part of the cerebellum and the caudal part of the mid-brain. The posterior cerebellar artery arises from about the mid-point of the basilar artery and runs laterally and dorsally around the medulla. It supplies small branches to the under-surface of the cerebellum. Caudal to this, the basilar often gives off another large lateral branch which does not, however, reach the cerebellum. There are three important branches of the vertebral artery. The most prominent of these is a longitudinal medullary branch (*L.A.*), which runs in a cranio-lateral direction, passing between the caudal rootlets of the hypoglossal nerve. It then runs cranially, lateral to the remaining hypo-

glossal rootlets and ends by joining either the posterior cerebellar artery or the branch lying caudal to it which was mentioned above. This longitudinal medullary artery gives off branches which run dorsally, the more caudal of which anastomose with branches of the posterior spinal artery. It may itself have two stems of origin from the vertebral. Each vertebral artery gives off a medially directed branch which unites with that of the opposite side to form the anterior spinal artery, which is still in a plexiform condition in the embryo which is illustrated. The third branch of the vertebral artery is the posterior spinal artery which arises from the dorsal aspect of the vertebral, has a marked cranially directed loop and then runs down to the spinal cord ventral to the dorsal nerve roots. The posterior spinal artery may take origin from the longitudinal medullary branch, or may have two roots, one from the vertebral and one from the longitudinal medullary artery.



Text-fig. 1. The hind-brain of an embryo in which the arteries illustrate stage 1 (semi-diagrammatic). The lateral longitudinal artery is connected to the longitudinal neural plexus by a number of very fine vessels and is continuous caudally with a longitudinal plexus on the side of the spinal cord. Five presegmental arteries are present, including a large otic artery. The row of small circles represents the attachment of the hypoglossal nerve rootlets.

The development of the hind-brain arteries during the period covered by this paper can conveniently be divided into three stages.

Stage 1. This stage is seen in embryos with a crown-rump length of from 4–6 mm., although these figures are only approximate since it is well known that there is considerable variation in the pattern of arteries between embryos of similar sizes. At this stage, the third, fourth and sixth aortic arches are present, as are the dorsal intersegmental arteries. The region of the future basilar artery is occupied by a pair of longitudinal plexuses which are connected across the mid-line by numerous transverse anastomoses, and which are fed cranially by the caudal branch of the primitive internal carotid and caudally by the ventral branch of the pro-atlantal artery (Text-fig. 1). In places, part of the plexus may be replaced by a single large vessel, but true longitudinal neural arteries extending the whole length of the hind-brain were never seen. These plexuses will therefore be referred to as the longitudinal

neural plexuses. They give off a number of small branches to the side of the brain, and two large vessels which are destined to become the anterior and posterior cerebellar arteries. The former arises at the cranial end of the hind-brain and runs dorsally around the isthmus. Its branches tend to turn caudally and their finer ramifications extend on to the thin roofplate of the hind-brain. The posterior cerebellar artery arises near the otocyst and runs dorsally before breaking up into fine branches.

The aorta and the primitive internal carotid artery are connected to the longitudinal neural plexus in the hind-brain region by a series of extremely interesting transverse communications (Pl. 1, fig. 2). There are usually three or four of these presegmental arteries, but as many as six on each side have been observed. Certain of these are constant in position while others appear to be variable. There is always a large presegmental artery in the vicinity of the otic vesicle, corresponding to the otic artery of Padget (1948). This vessel arises from the dorso-medial aspect of the primitive internal carotid opposite the second arch, and frequently joins the longitudinal neural plexus at the point where that plexus gives off the posterior cerebellar artery. Often, however, the latter artery arises a little more caudally and in this case there is a second presegmental vessel lying caudal to the otic artery and opening into the longitudinal neural plexus opposite the origin of the posterior cerebellar artery. In some cases, both these presegmental arteries are present, the otic artery being continuous through the longitudinal neural plexus with the posterior cerebellar artery, and the other presegmental artery similarly becoming continuous with a rather smaller vessel running parallel with, and caudal to, the posterior cerebellar. The latter vessel is usually joined by a lateral longitudinal artery which will be described later. In one embryo the more caudal of these two presegmental vessels by-passed the longitudinal neural plexus altogether and ran straight from the primitive internal carotid to the side of the brain where it joined the lateral longitudinal artery. In another embryo the arrangement was similar, but there was a very small communication with the longitudinal neural plexus.

There is often a large presegmental artery in the region of the trigeminal nerve, but this does not seem to be as constant or as important as the trigeminal artery described by Padget (1948) in human embryos. In the region of the rootlets of the hypoglossal nerve there may be one or more presegmental arteries, but these never attain any great size and are not as constant in position as one is led to believe from the literature on the 'hypoglossal artery' in the human.

The vessel which lies cranial to the first cervical nerve will here be referred to as the pro-atlantal intersegmental artery in accordance with Padget's (1954) nomenclature. This vessel takes origin from the dorsal wall of the aorta a little caudal to the sixth arch and divides into dorsal and ventral branches. The ventral division turns medially and cranially to join the caudal end of the longitudinal neural plexus of the same side. The dorsal branch runs on to the side of the hind-brain, gives off a large cranial and a smaller caudal branch and is then expended in small branches which ramify on the dorsal part of the brain. The most important of all these branches is the one which runs cranially, and this is so large that it gives the appearance of being the main continuation of the pro-atlantal artery (Pl. 1, fig. 1). It lies lateral to the rootlets of the hypoglossal nerve and medial to the rootlets of the glosso-

pharyngeal, vagus and accessory nerves and gives off dorsal branches to supply the hind-brain. This vessel corresponds to the primitive lateral basilo-vertebral anastomosis of Padget (1948), but as will be explained later it is more than an anastomotic channel in the rat, and will here be referred to as the lateral longitudinal artery. Its cranial end may break up into branches, but more commonly it anastomoses directly with the posterior cerebellar artery or with the smaller vessel which has already been described as lying caudal to that artery. During the injection, the lateral longitudinal artery is one of the first vessels in the hind-brain region to fill, and it is always prominent, even in imperfectly injected specimens. There are numerous extremely fine hair-like vessels connecting the lateral longitudinal artery to the longitudinal neural plexus.

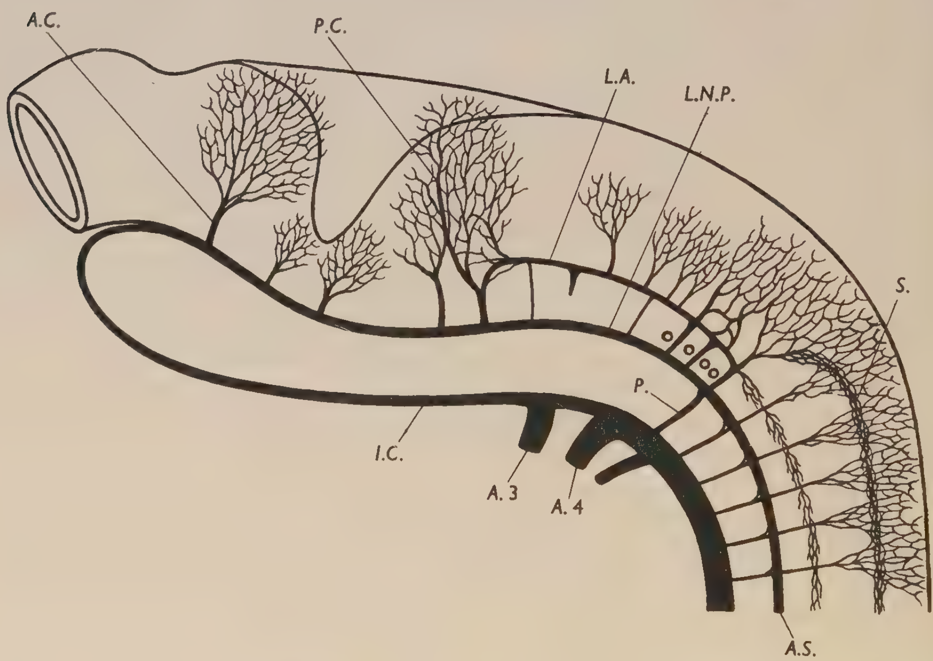
The caudal branch of the pro-atlantal artery forms the cranial end of a longitudinal anastomosis between the dorsal intersegmental arteries which lies on the side of the spinal cord. This anastomosis may be in the form of a continuous vessel or, more commonly, a plexus, and it is important to note that it is directly in line with the lateral longitudinal artery so that the appearance is given of a continuous channel running up the side of the spinal cord and along the hind-brain. The remaining intersegmental arteries pass dorsally to the side of the spinal cord, giving a ventral branch to the anterior spinal plexus of the corresponding side. They then give off cranial and caudal branches which help to form the longitudinal anastomosis on the side of the spinal cord, and finally break up into small branches which form a plexus on the dorsal part of the spinal cord. The plexus stops short of the mid-line, but there are numerous fine transverse vessels which join the plexuses of the two sides.

Stage 2. This stage is represented by embryos with crown-rump lengths of between 6 and 8 mm., and is illustrated in Text-figs. 2 and 3, and Pl. 1, fig. 3. The longitudinal neural plexuses, together with the transverse anastomoses which connect them, have now enlarged to form a single wide mid-line plexus on the ventral surface of the hind-brain, but the appearance is such that it is difficult to know whether to describe it as a dense plexus composed of wide vessels or a single large mid-line vessel with numerous 'Inselbildungen'. The caudal end of the plexus, however, retains its original bilateral character and is fed by the ventral branch of the pro-atlantal artery. It is continuous with the much smaller bilateral plexus which will later form the anterior spinal artery.

The presegmental vessels are no longer present. They disappear a little after the 6 mm. stage, the vessel or vessels in the region of the otic vesicle being usually the last to go.

The dorsal branch of the pro-atlantal artery still gives origin to the large lateral longitudinal artery, before breaking up into a number of fine branches on the side of the hind-brain. The original longitudinal anastomosis on the side of the spinal cord, however, has been replaced by a longitudinal plexus which lies more dorsally, although remnants of the original anastomosis were seen in three embryos (Text-fig. 2). The new plexus lies immediately ventral to the dorsal nerve roots and will become the posterior spinal artery. Cranially, it communicates with the dorsally directed branches of the pro-atlantal artery. It also receives contributions from the other intersegmental arteries which, after giving off a ventral branch to the anterior

spinal plexus, break up into a leash of fine vessels on the side of the spinal cord and eventually reach the posterior spinal artery. Occasionally one particularly large vessel is seen to run through this network to reach the posterior spinal artery directly. This occurs particularly in the case of the upper three intersegmental arteries, although it must be remembered that dissections were only made down to the level of the subclavian artery. Branches of the intersegmental arteries other than the neural branches were removed during dissection and will not be described.

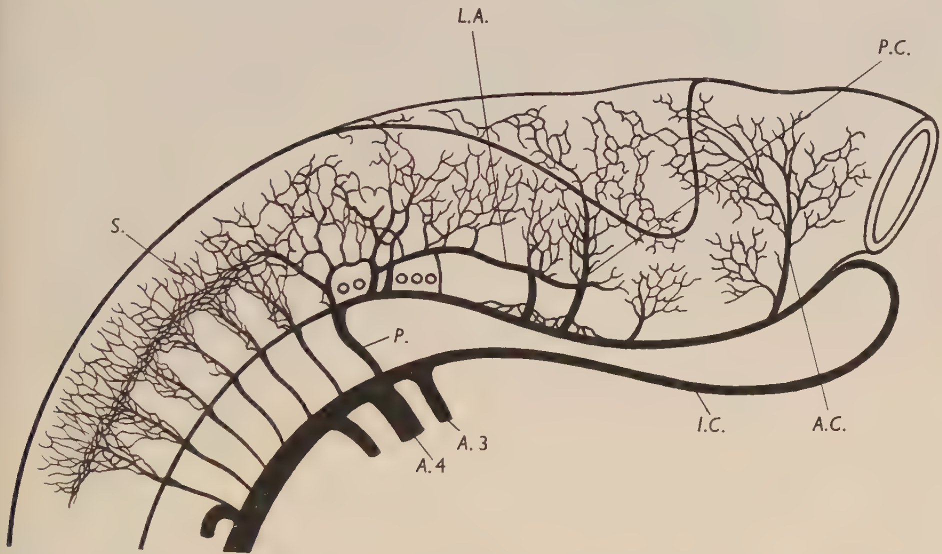


Text-fig. 2. A 6·5 mm. embryo in the early part of stage 2 (semi-diagrammatic). The presegmental arteries have disappeared, and there are large communications between the lateral longitudinal artery and the longitudinal neural plexus. A plexus has appeared in the region of the posterior spinal artery and remnants of the original longitudinal plexus on the side of the spinal cord can still be seen.

The lateral longitudinal artery is still very prominent, and gives off a number of branches to the lateral and dorsal parts of the hind-brain. The numerous fine vessels which were previously seen to be connecting the lateral longitudinal artery to the longitudinal neural plexus have now been replaced by a smaller number of much larger vessels. They vary in number from three to five, the most cranial of these forming the stem of origin of the posterior cerebellar artery or of the vessel caudal and parallel to that vessel. The most caudal one or two of these anastomoses between the longitudinal neural plexus and the lateral longitudinal artery are particularly large and pass between the rootlets of the hypoglossal nerve. Towards the end of the second stage, one or both of these vessels take over the lateral longitudinal artery from the pro-atlantal, the more caudal part of the lateral longitudinal artery disap-

pearing (Text-fig. 3; Pl. 1, fig. 3). This artery thus becomes a branch of the caudal (unfused) part of the longitudinal neural plexus and passes between the hypoglossal rootlets in the first part of its course.

One frequently sees embryos which are intermediate between the first and second stages and which have both presegmental arteries and large anastomoses between the longitudinal neural plexus and the lateral longitudinal artery. These anastomoses usually lie directly opposite the presegmental arteries so that the latter appear to be passing directly from the aorta to the lateral longitudinal artery, communicating on the way with the lateral edge of the longitudinal neural plexus.

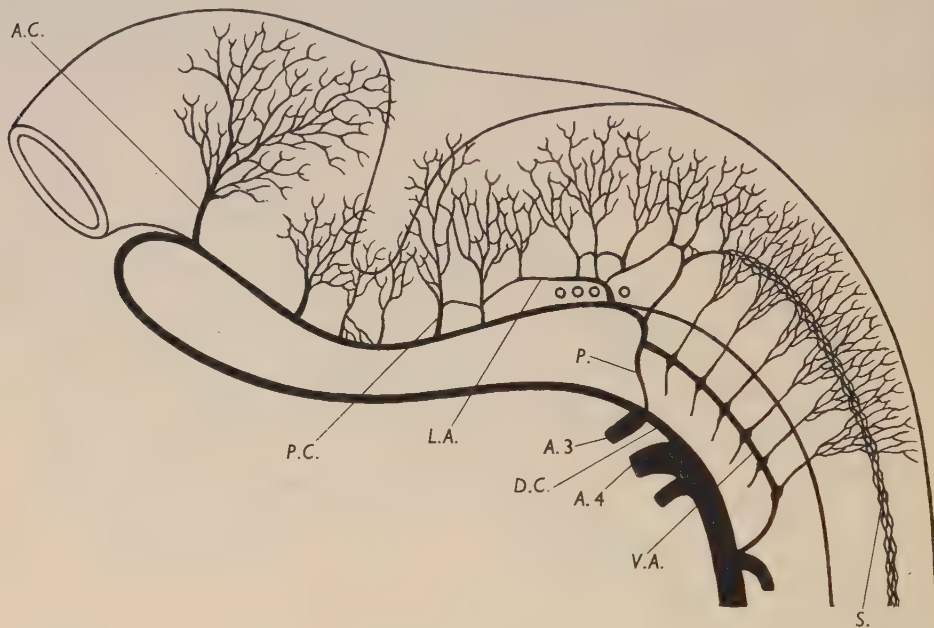


Text-fig. 3. A 7·8 mm. embryo towards the end of stage 2 (semi-diagrammatic). The origin of the lateral longitudinal artery from the pro-atlantal artery is diminishing in size so that the former vessel appears to arise by two roots from the caudal part of the longitudinal neural plexus, and passes between the hypoglossal nerve rootlets.

Stage 3. This stage is seen in embryos of 9 mm. and more in length and roughly corresponds to the adult configuration. The ductus caroticus is absent, or reduced to a fine thread, and the pro-atlantal artery arises opposite the third aortic arch.

The basilar artery is a single mid-line vessel but still shows in places some signs of its original plexiform nature (Pl. 2, fig. 6). The vertebral artery is now present, its cranial end being formed by the ventral branch of the pro-atlantal artery together with, as will be shown later, the caudal unfused part of the longitudinal neural plexus. With the formation of the vertebral artery, the more proximal part of the intersegmental arteries disappears, although one can frequently see a very thin remnant of the proximal part of the pro-atlantal artery after the others have gone (Pl. 2, fig. 4). The dorsally directed branches of the intersegmental arteries persist as branches of the vertebral artery which form a plexus on the side of the spinal cord and the ventral surfaces of the posterior root ganglia before communicating with the posterior spinal artery. The cranial root of the latter vessel is now usually

formed by the dorsal branch of the pro-atlantal artery so that in adult terminology, the posterior spinal is a branch of the vertebral artery (Pl. 2, fig. 5). Occasionally, however, the dorsal branch of the pro-atlantal artery may regress, leaving the posterior spinal artery to obtain its cranial blood supply from one of the dorsal branches of the lateral longitudinal artery (Pl. 2, fig. 4).



Text-fig. 4. A 9·5 mm. embryo at stage 3 (semi-diagrammatic). The vertebral artery has now formed and the dorsal parts of the intersegmental arteries have become branches of that artery. The intersegmental arteries, except for the pro-atlantal, have lost their origin from the aorta. The intracranial portion of the vertebral artery gives off the posterior spinal artery and the lateral longitudinal artery, the latter passing between the hypoglossal rootlets.

The lateral longitudinal artery is now a branch of the intracranial part of the vertebral artery and passes laterally between the hypoglossal rootlets. It thus forms the longitudinal medullary artery of the adult. One can therefore say that at this stage the adult pattern has been reached except that the anterior spinal artery is still in the form of a bilateral plexus and that the cerebellum is still in the early stages of its development so that the anterior and posterior cerebellar arteries do not have their adult distribution.

DISCUSSION

The study of the development of the vascular system by the method of injection has much to commend it. It is easy to perform after a little practice and requires no elaborate apparatus. It enables a large number of embryos to be studied in a comparatively short time so that variations in the normal pattern are recognized as such and, finally, an accurate picture of the embryonic vascular plexuses is obtained.

Even incompletely injected specimens may be of use, since as a 'physiological injection' is produced the parts of the vascular system which are filled with ink are

presumably those parts which are most advantageously situated with regard to their blood supply. Observations made during the actual injection are also helpful and in this respect it is interesting to note that the lateral longitudinal artery is one of the first vessels to fill after the aortic arches and dorsal aorta. It is often rather difficult to obtain a complete injection of the region of the pontine flexure since this portion of the embryonic brain lies in the boundary zone between a caudally directed and a cranially directed blood flow. The former is derived from the primitive internal carotid and the latter from the pro-atlantal artery and the lateral longitudinal artery. Thus, the vessels of the fore- and mid-brains usually fill easily as do the intersegmental arteries, but the only vessel in the hind-brain region which can be seen easily in the early stages of the injection is the caudal portion of the lateral longitudinal artery, and one often has to wait for some time before the ink reaches the region of the pontine flexure.

In the earlier stages studied in this investigation, the presegmental arteries are of particular interest. Few authors, however, have studied them in detail and they appear to be subject to a good deal of variation in different species.

The original appearance of these vessels takes place at an earlier period than that covered by the present work, since they first develop as direct neural branches of the dorsal aorta before the longitudinal neural arteries have formed. Evans (1912), who found presegmental vessels in three early human embryos, stated that 'they represent the first vascular sprouts sent out by the dorsal aorta into the tissues of the embryo in this region and, directed towards the side of the medullary tube, are directly responsible for the formation of the vena capitis medialis'. Similar vessels have been described by Sabin (1917) and Streeter (1918). Since the present series does not cover these early stages I shall only deal here with the presegmental arteries as they appear when fully formed as branches running from the aorta or primitive internal carotid artery to the longitudinal neural plexus.

The most cranially situated of these vessels was found to lie in the vicinity of the trigeminal nerve and was usually, but not invariably present. A similar vessel has been described by de Vriese (1905) and Fuchs (1905) in rabbit embryos. Tandler (1902) described a row of six presegmental vessels lying cranial to the pro-atlantal artery in the rat, but gives little information about them. In his fig. 8, however, the most cranial of these arteries lies opposite the first arch, and a similar vessel is seen in his fig. 14. Padget (1948) has given a detailed account of this 'trigeminal artery' in human embryos and stresses its importance in supplying the longitudinal neural arteries in the early stages of their formation. In the rat, however, this vessel seems to be less important than some of the other presegmental arteries.

There was always found to be at least one large presegmental artery in the region of the otic vesicle. This vessel arose opposite the second arch and usually opened into the longitudinal neural plexus immediately opposite the origin of the posterior cerebellar artery. In those cases in which there were two large presegmental vessels in this region, the posterior cerebellar artery usually arose opposite the more caudal of the two. The otic artery has been mentioned by several previous authors. De Vriese (1905) found a relatively constant presegmental vessel in this region in the rabbit, while Sabin (1917), in her plate 7 shows two such vessels in a 6·5 mm. pig embryo. Padget (1948) found two or three slender communications between the

dorsal aorta and the longitudinal neural arteries near the otocyst in a 4 mm. human embryo, while in a more advanced embryo of the same length there was only one small communication on the left side. Tandler (1902) describes a presegmental vessel arising opposite the second aortic arch with another lying caudal to it, but unfortunately no further details are given. In the present series, the otic artery or arteries were the largest and least transitory of all the presegmental vessels and seem to be as important in the blood supply of the longitudinal neural plexuses in the early stages as are the trigeminal arteries in the human.

The presegmental vessels in the region of the hypoglossal nerve seem to be particularly subject to variation and there are many brief and incomplete references to them in the literature. Froriep (1886) mentions the presence of 'interprotovertebral arteries' in the occipital region in chick embryos but was unable to find any in the cow. Dawes (1930), in *Mus musculus*, found five rudimentary segments in the occipital region and states that intersegmental arteries pass between these segments and in front of the first, but he gives no details of their distribution. Hochstetter (1890) described a hypoglossal artery in 11-day rabbit embryos, and de Vriese (1905), also in the rabbit, found a relatively constant vessel in this region. Tandler (1902) found a similar artery in both rat and human embryo; the presence of this vessel in the human has also been mentioned by Zimmerman (1889), Ingalls (1907), Thyng (1914), Johnson (1917), Congdon (1922), Schmeidel (1932) and Padget (1948). According to Tandler (1902) and Ingalls (1907), the hypoglossal artery for a time forms the caudal root of the 'cerebral vertebral artery' (i.e. the longitudinal neural artery), which later becomes taken over by the pro-atlantal artery. I have been unable to confirm this since, in all the embryos studied, the ventral branch of the pro-atlantal artery was large and formed the main caudal blood supply to the longitudinal neural plexus. It may well be that the present series does not include embryos which are sufficiently young, although in Tandler's series of rat embryos the pro-atlantal artery did not communicate with the longitudinal neural arteries until the 5·5–6 mm. stage.

Schmeidel's account of these vessels is the most detailed. He found from one to three hypoglossal arteries in human embryos of 5·49, 4·53, 5·98 and 8 mm. In his embryo Sz2, the hypoglossal artery passed between the hypoglossal rootlets to join the lateral longitudinal artery without communicating with the longitudinal neural artery. It was the persistence of such an artery that was believed to be responsible for the anomalous basilar artery previously described (Morris & Moffat, in the press). It is interesting that a similar direct communication between the primitive internal carotid artery and the lateral longitudinal artery was found in the present series, lying just caudal to the posterior cerebellar artery, but no such vessel was found in the hypoglossal region.

The hypoglossal arteries in the rat embryo studied in the present series were inconstant in position and number. The communications between the longitudinal neural plexus and the lateral longitudinal artery were large and extremely important, but the true hypoglossal arteries were always very slender vessels and were sometimes missing altogether, even though the other presegmental vessels were fully formed.

For the sake of completeness, it must be mentioned that two further authors have

referred to presegmental vessels without describing their position. Sicher (1912) found two such vessels in *Talpa europaea*, and von Hofman (1914) found two presegmental arteries in the pig.

The presegmental vessels are sometimes referred to as 'cranial intersegmental arteries'. I think that more evidence as to their nature is needed before this term can justifiably be used. In the embryos which have been mentioned as being intermediate in appearance between stages 1 and 2, the presegmental arteries appeared to pass from the dorsal aorta to the lateral longitudinal artery, communicating between these two vessels with the longitudinal neural plexus. It is tempting, in these cases, to compare the presegmental arteries to the ordinary dorsal intersegmental arteries and to regard them as giving a ventral branch to the longitudinal neural plexus and a dorsal branch to the lateral longitudinal arteries. Schmeidel (1932) wrote of the communications between the longitudinal neural arteries and the lateral longitudinal artery: 'Diese arteriellen Verbindungen zwischen den beiden Längsgefässen stellen, wie ich glaube, die peripheren Stücke der Hypoglossusarterien dar....' There is no doubt, however, that the true presegmental vessels appear before the anastomoses between the longitudinal neural plexus and the lateral longitudinal arteries, and the fact that these anastomoses, when they finally appear, lie opposite the presegmental vessels may well be a hydrodynamic effect. In much the same way, the larger dorsal branches of the lateral longitudinal artery often lie opposite the anastomoses between that vessel and the longitudinal neural plexus. A further point against likening the presegmental to the dorsal intersegmental arteries is the fact that, as Tandler (1902) has mentioned, the former vessels arise from the medial wall of the aorta rather than from its dorsal aspect.

One point which is well brought out by the injection technique concerns the importance of the presegmental vessels in the supply of blood to the longitudinal neural plexuses. It is frequently stated that the latter vessels are fed mainly by the caudal branch of the internal carotid artery and by the hypoglossal or pro-atlantal arteries, although Strong (1947). who worked on injected rabbit embryos, stated that they develop from anastomoses between the 'carotid intersegmentals'. According to Congdon (1922) they 'probably arise as anastomoses between all segmental arteries cranial to the second cervical, followed by a loss of the connexion of these vessels with the aorta'. Padget (1948), in human embryos of 4–5 mm., found that the main cranial supply to the longitudinal neural arteries was the trigeminal artery, reinforced to a variable extent by remnants of the 'highly transitory otic and hypoglossal arteries'.

Pl. 1, fig. 2, shows a ventral view of the hind-brain region of a 5·5 mm. embryo with three presegmental vessels on each side passing from the dorsal aorta to the longitudinal neural plexus. It will be seen that the plexus is only well injected cranially, where it is fed by the primitive internal carotid, caudally where it receives blood from the pro-atlantal artery and, between these two points, in the regions where it is joined by the presegmental arteries. In other embryos, the longitudinal neural plexuses could only be seen as a series of plexiform islands in these situations, the intervening portions being uninjected. A very similar appearance is seen in Padget's (1948) plate 4, fig. 39. Whether this implies that vessels grow cranially and caudally from each of these foci before linking with one another to form the longitudinal

neural plexus, or whether the appearance was simply the result of incomplete injection is a matter which cannot be decided by the present technique, but in the light of the findings of previous authors it seems likely that the former supposition is the correct one. In any case, the injection technique does indicate that the pre-segmental vessels are extremely important in supplying blood to the longitudinal neural plexus at this stage.

It is surprising that so few authors have mentioned the lateral longitudinal artery. Elze (1907) found this vessel in human embryos of 7, 9·5 and 11 mm., describing it as a longitudinal anastomosis between a number of lateral branches of the longitudinal neural arteries. Barniville (1914) also described it as an 'irregular longitudinal anastomosis' which he found in an 8·5 mm. human embryo. Padget (1948) calls it the 'primitive lateral basilo-vertebral anastomosis' and found it to be fed cranially by lateral branches of the basilar artery and caudally by the pro-atlantal artery. Schmeidel (1932) has recognized the fact that this vessel is essentially a cranially directed branch of the pro-atlantal artery, but in all his embryos it was also fed by lateral branches of the basilar artery, these branches passing between the hypoglossal rootlets. The illustration which most closely resembles conditions in rat embryos is that of Heuser's (1923) plate 2, fig. 15. This shows a 12 mm. pig embryo prepared by the injection technique and indicates that the lateral longitudinal artery is derived from the pro-atlantal artery, and this diagram illustrates almost exactly the appearance seen in the early stages of the injection of a rat embryo of about 4–6 mm. in length. Only a very brief mention of the lateral longitudinal artery is made in the text, but Heuser does mention that large anastomoses later form between this vessel and the primitive basilar artery. The present study also indicates that the lateral longitudinal artery is present before the formation of the large laterally directed branches of the longitudinal neural plexus. It is therefore important to realize that, at least in the rat, the lateral longitudinal artery is not a longitudinal anastomosis between these laterally directed branches, but is a primary vessel which later acquires anastomoses with the longitudinal neural arteries. The replacement of the origin of the lateral longitudinal artery from the pro-atlantal artery by the more caudal of the anastomoses between the longitudinal neural plexus and the first-named vessel has not been mentioned before, probably because in human embryos the lateral longitudinal artery is a temporary structure. However, in Schmeidel's embryo P, of 11 mm., the lateral longitudinal artery had apparently lost its connexion with the pro-atlantal artery.

The significance of the longitudinal anastomosis on the side of the spinal cord with which the lateral longitudinal artery is continuous is not clear. It is often plexiform, and sometimes incomplete, but its continuity with the lateral longitudinal artery and the resulting appearance of a continuous longitudinal channel running up the spinal cord and along the side of the hind-brain is most striking. It seems probable that this anastomosis is a remnant of the original longitudinal anastomosis between the dorsal intersegmental arteries which is found at a very early stage. The appearance is very similar to that seen in a 30-somite chick embryo depicted by Evans (1909) in his fig. 1, except of course for the fact that in rat embryos at this stage somewhat smaller vessels have spread ventrally to form the anterior spinal plexus and dorsally on to the dorsolateral surface of the spinal cord. In Heuser's

(1923) fig. 15, mentioned above, part of a similar longitudinal anastomosis can be seen connecting the third, fourth, fifth and sixth dorsal intersegmental arteries, and it is clear that were it continued cranially it would be continuous with the lateral longitudinal artery. The longitudinal anastomosis is quite distinct from the posterior spinal artery, since the latter is situated considerably dorsal to the line of the lateral longitudinal artery and, in three embryos, remnants of the longitudinal anastomosis could be seen at a stage when the definitive posterior spinal artery was present (Text-fig. 2).

Finally, the formation of the basilar artery must be mentioned. Most previous authors state that it is formed from two ventral longitudinal vessels (the bilateral longitudinal neural arteries) by their fusion or by the persistence of alternate right and left segments. Congdon (1922), however, has pointed out from a review of previous work that 'the longitudinal neural artery in mammals varies from a zone of enlarged vessels in a plexus to a single continuous channel'. In the rat, Tandler (1902) described a longitudinal neural artery but I have never seen such a vessel running the whole length of the hind-brain. Before the definitive basilar artery is formed, the base of the hind-brain is occupied by a bilateral longitudinal plexus of vessels connected across the mid-line by numerous transverse anastomoses. Occasionally a single larger vessel can be seen running through the plexus, particularly in the vicinity of the larger presegmental vessels, but this single vessel is present only for a short distance. Stage 2 embryos (6–8 mm.) show enlargement of the vessels of the plexus and of the transverse anastomoses, so that a wide strip of the ventral surface of the hind-brain is covered by a dense plexus whose meshes are so small that one could call it a wide fenestrated mid-line vessel. By the 9 mm. stage this has become the basilar artery which frequently shows evidence of its plexiform origin, as can be seen in Pl. 2, fig. 6. Congdon (1922) was uncertain whether a portion of the caudal end of the longitudinal neural artery remains unfused to form the distal end of the vertebral artery. In the rat, the stem of origin of the lateral longitudinal artery provides a useful landmark. Since the definitive origin of this vessel consists of one of the anastomoses between the longitudinal neural plexus and the lateral longitudinal artery, and since the corresponding vessel in the adult arises from the vertebral artery, it becomes clear that the terminal portion of the vertebral artery is derived from the longitudinal neural plexus.

SUMMARY

1. The development of the hind-brain arteries has been studied in a series of dissections of injected rat embryos.

2. In stage 1 (4-6 mm. approx.), the base of the hind-brain is occupied by a pair of longitudinal neural plexuses which are fed by the primitive internal carotid, the pro-atlantal artery and a series of presegmental arteries. The pro-atlantal also gives rise to a lateral longitudinal artery which lies lateral to the hypoglossal rootlets and which is continued caudally as a longitudinal anastomosis between the dorsal intersegmental arteries along the side of the spinal cord. The anterior and posterior cerebellar arteries can already be recognized.

3. In stage 2 (6–8 mm. approx.), the presegmental arteries disappear and the longitudinal neural plexuses coalesce to form the basilar artery. A number of large

anastomoses develop between the lateral longitudinal artery and the basilar artery or the caudal unfused part of the longitudinal neural plexus. The lateral longitudinal artery then loses its origin from the pro-atlantal artery and becomes a branch of the caudal part of the longitudinal neural plexus. The dorsal branch of the pro-atlantal artery becomes the stem of origin of the posterior spinal artery.

4. In stage 3, which approximates to the adult condition, the cervical part of the vertebral artery has developed as an anastomosis between the dorsal intersegmentals, its terminal portion being formed by the ventral branch of the pro-atlantal artery and the caudal part of the longitudinal neural plexus. The posterior spinal and lateral longitudinal arteries thus become branches of the vertebral artery.

5. These findings are compared with those of previous workers, with particular reference to the presegmental arteries, the lateral longitudinal artery and the basilar artery.

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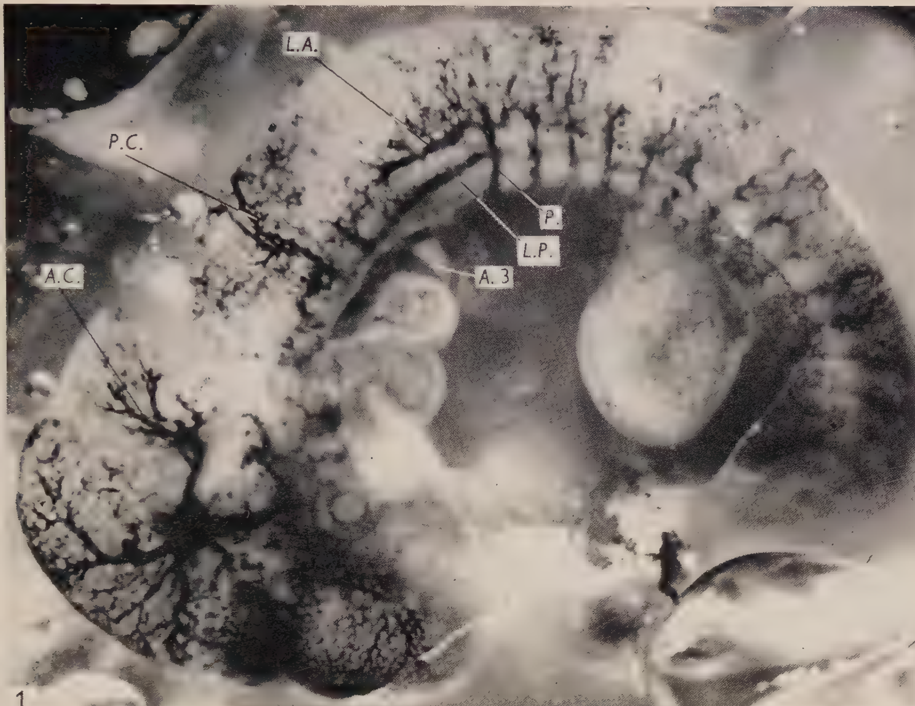

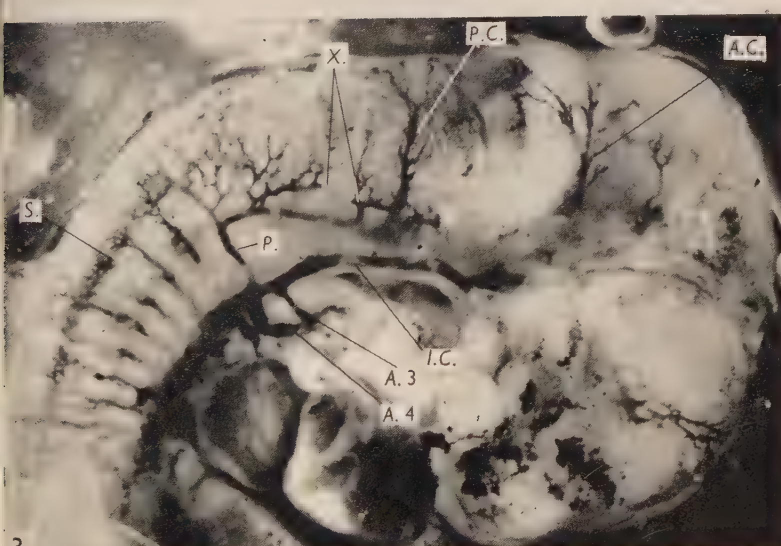

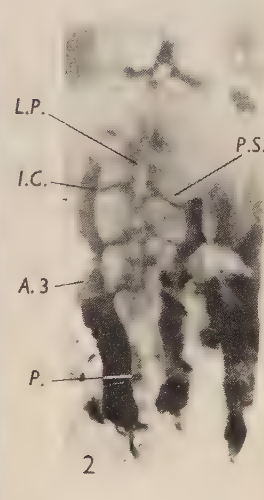

MOFFAT—DEVELOPMENT OF THE HIND-BRAIN ARTERIES IN THE RAT

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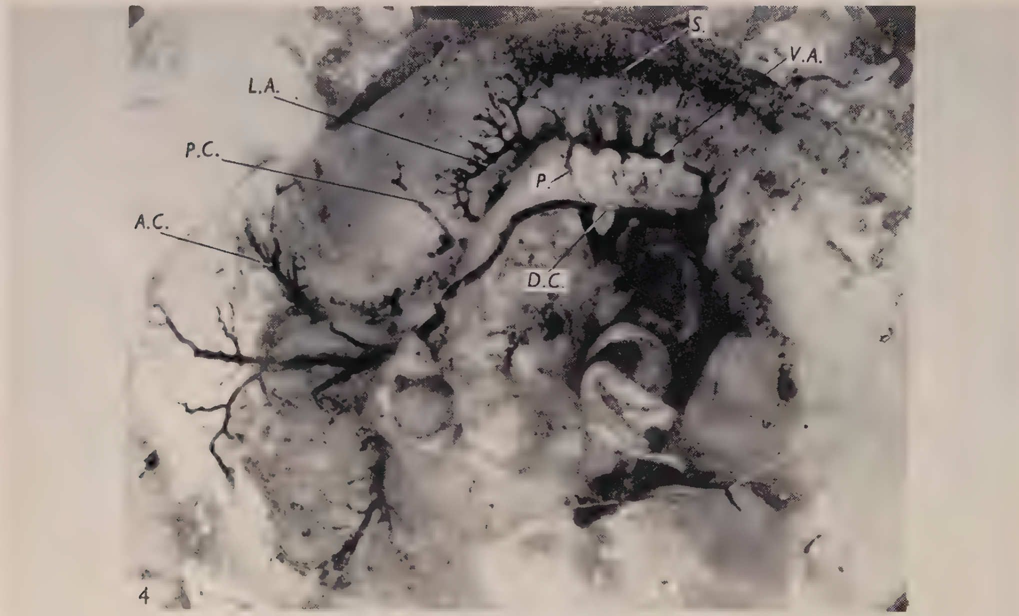

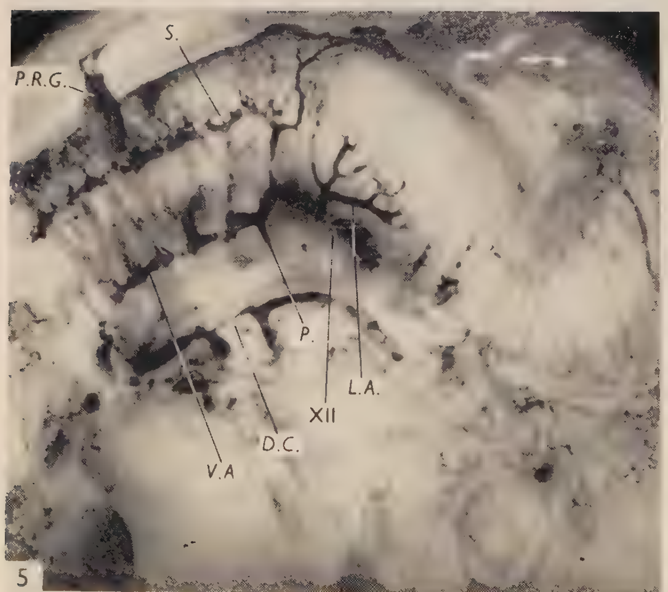

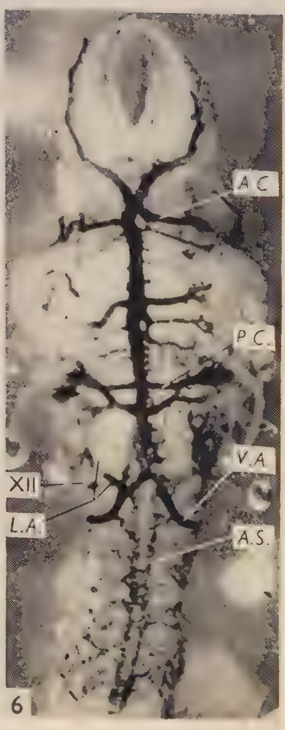

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KEY TO ABBREVIATIONS

A3	Third aortic arch
A4	Fourth aortic arch
A.C.	Anterior cerebellar artery
A.S.	Anterior spinal artery
D.C.	Ductus caroticus
I.C.	Internal carotid artery
L.A.	Lateral longitudinal artery
L.P.	Longitudinal neural plexus
P.	Pro-atlantal artery
P.C.	Posterior cerebellar artery
P.S.	Presegmental artery
P.R.G.	Posterior root ganglion
S.	Posterior spinal artery
V.A.	Vertebral artery
XII	Rootlets of hypoglossal nerve

EXPLANATION OF PLATES

Plate 1

Fig. 1. The left side of a 6·2 mm. embryo to illustrate stage 1, in which the lateral longitudinal artery is connected to the longitudinal neural plexus by a number of very fine vessels. A strip of tissue containing the trigeminal nerve has been left *in situ*. Three presegmental vessels were present but are not visible in this view.

Fig. 2. Ventral aspect of the hind-brain region of a 5·5 mm. embryo to show the presegmental arteries and the longitudinal neural plexus.

Fig. 3. A 7·8 mm. embryo, towards the end of stage 2. The presegmental arteries have disappeared and the origin of the lateral longitudinal artery from the pro-atlantal is very small. A plexus has now appeared in the position of the posterior spinal artery. A small part (*X*) of the lateral longitudinal artery was damaged during the dissection.

Plate 2

Fig. 4. A dissection of the region of the vertebral artery in a 9·4 mm. embryo. The dorsal branch of the pro-atlantal artery has almost disappeared, so that the posterior spinal artery will become a branch of the lateral longitudinal artery. The third aortic arch has been slightly retouched.

Fig. 5. The opposite side of the same embryo. The dorsal branch of the pro-atlantal artery forms the stem of origin of the posterior spinal artery. A posterior root ganglion has been turned back to show the vessels on its ventral aspect.

Fig. 6. Ventral aspect of the hind-brain in a 9·5 mm. embryo. This shows the adult pattern of vessels except that the anterior spinal artery is not yet in the mid-line. The basilar artery still shows signs of its origin from a plexus.

THE FORM AND DEVELOPMENT OF THE BLOOD VESSELS OF THE MAMMALIAN CEREBRAL CORTEX

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Pfeifer (1930, 1940) and Cobb (1931, 1932) have described two types of vessels arising in the pia mater and entering the cerebral cortex—short perforating cortical vessels terminating in the cortex and long perforating cortico-medullary vessels, traversing the cortex and terminating in the white matter. They also described deep medullary vessels, possibly arising from the perforating basal arteries, approaching the cortex from below and joining its capillary bed. All these vessels were described as arborizing to form a continuous capillary network. Sunderland (1938) reached similar conclusions, but doubted the existence of the deep medullary vessels, whilst Scharrer (1940) showed that Pfeifer had described arteries as veins and veins as arteries. This pattern has been found to be characteristic of eutherian mammals; a similar net-like system of vessels has been described in the monotremes (Sunderland, 1941), the snakes and Chelonia, the Anura, and most fish.

Wislocki & Campbell (1937) have described the unusual form of the blood vessels in the opossum brain, in which an artery and a vein arborize together, their terminal branches being joined by hairpin-like capillary loops. Other marsupials were found by Sunderland (1941) to possess a similar pattern, and the same type of vascularization has been described in most lizards. The brains of urodele amphibia were said to be supplied by simple hairpin-like capillary loops entering from the surface and thus forming a rather similar, though simpler, pattern to that of the opossum brain. The same looped pattern has been described in the brain of the lamprey, and even in the nervous system of certain invertebrates, e.g. the earthworm (Scharrer, 1944). Wislocki (1939) also showed that the typical vascular pattern of the opossum brain is an elaboration of a simple looped system in the embryo which is comparable to that of the adult urodele amphibian brain. Vascular arrangements intermediate between the net-like and the looped patterns have been described in the lungfish *Epiceratodus* (Craigie, 1943) and in the salamander *Ambystoma*. Here occasional hairpin loops were found interspersed among a predominantly reticular pattern. These various vascular patterns have been reviewed by Craigie (1938, 1945, 1955) who stressed the fact that their phylogeny was obscure.

The development of the vessels of the central nervous system has been studied by Williams (1937) and Feeney & Watterson (1946) who described radial vessels penetrating the neural tube and linking up to form an endoneural plexus on the surface of the ependymal layer, which extended peripherally in the developing neural tube by the formation of cross-anastomoses between the radial vessels.

The view has been held that the looped form was primitive and the reticular pattern derived from it, but as Craigie (1938) and Sunderland (1941) pointed out,

there has been no evidence produced indicating a transformation of one type to the other either phylogenetically or ontogenetically.

The purpose of the present investigation was to see whether embryonic higher mammalian brains possessed a vascular bed comparable to the simple looped patterns of vascular supply, thus indicating a derivation of the reticular from the looped pattern in phylogeny.

METHODS

The brains of rabbit embryos of 16–26 days gestation, injected with 10 % Monastral Fast Blue BNVS paste were sectioned at 400μ thickness and the sections cleared and mounted. Similar sections of the brain of an adult rabbit, a foetal sheep (C.R. 22 cm.), 55 and 90 mm. guinea-pig embryos, 17 and 24 mm. rat embryos, a human foetus (C.R. 12·5 cm.), and a crested newt, all injected with Monastral Fast Blue, were prepared. The brains of four rabbit embryos, injected with Monastral Fast Blue, were also dissected under the binocular microscope.

OBSERVATIONS

Sections of the cerebral hemisphere of an injected 16-day rabbit embryo show it to be vascularized by radially penetrating vessels arising from the pia mater and joining a plexus in the deeper parts of the wall (Pl. 1, fig. 6).

Cross-anastomoses between the radial vessels develop so that sections of the hemispheres of the injected brain of a 21-day rabbit embryo show that the cortex is supplied from the pia mater by a series of capillary loops which are connected together so that a system of vascular arcades of one, two or three orders, is built up, though a basic-looped pattern remains clearly apparent. Among the arcades are occasionally found simple hairpin-like loops penetrating from the pia mater. These are similar to those of urodele amphibian brains, though here a branch may arise from one limb of the loop and join the arcade system (Pl. 2, figs. 12, 13). Short loops of similar form may arise from the vessels forming the arcades. Large vessels traverse this system perpendicularly to the surface and break up into a net-like arborization in the subjacent white matter. Some of these perforating cortico-medullary vessels are veins which are joined at about right angles by several tributaries as they traverse the cortex and present a knotty appearance due to dilatations at these points of union. Other are perforating cortico-medullary arteries and these present a more regular lumen with generally no branches as they traverse the cortex except for an occasional branch joining the arcade system in the deepest part of the cortex. A fairly evident, rather avascular line of separation exists between the cortical and medullary capillary beds and branches of the long perforating cortico-medullary arteries and veins often run along this plane, parallel to the surface, before entering the white matter; such vessels also give occasional small branches passing superficially to join the cortical arcades, and other capillary connexions are also quite common between the two systems across the separation line (Pl. 1, figs. 1, 2).

In a 23-day rabbit embryo the vascularity of the thickening cortex is increasing by the development of many cross-anastomoses between the radially arranged limbs of the original loops and arcades, giving rise to an appearance of arcades of many

orders. Otherwise the vascular pattern is unchanged from the earlier stage (Pl. 1, fig. 4).

A 26-day rabbit embryo shows this process to have progressed further and the cortex, with its radially running vessels connected by cross-anastomoses, is now quite definitely more vascular than the underlying white matter with its capillary net supplied by the long perforating cortico-medullary arteries and drained by the perforating veins. At this stage some of the radial channels of the cortex are commencing to develop into more definite arterioles and venules (Pl. 1, fig. 3).

The injected brain of an adult rabbit shows a further elaboration of the 26-day embryo stage. Many more cross-anastomoses developed in the cortical system, together with elaboration of more arteries and veins from its radial channels, have rather obscured the arcade pattern (Pl. 2, fig. 10). Moreover, in some places vessels are now seen approaching and joining the deep aspect of the cortical capillary bed, through the comparatively avascular white matter (Pl. 2, fig. 9). These would at first sight appear to be deep medullary arteries as described by Pfeifer and by Cobb. However, if followed they are seen to be branches arising from the long perforating cortico-medullary arteries or veins after these have entered the medullary substance and which turn back superficially to join the deep aspect of the cortical capillary bed (Pl. 2, fig. 11). The largest of these recurrent vessels are veins.

The blood vessel patterns of 17 and 24 mm. rat embryo brains correspond in form to 16- and 21-day rabbit embryo brains and again here simple hairpin loops are occasionally found (Pl. 1, fig. 7).

A 55 mm. guinea-pig brain shows the same vascular pattern as a rabbit embryo of about 26 days and by 90 mm. has been elaborated almost to the form seen in an adult rabbit.

The human foetus (C.R. 12·5 cm.) and sheep embryo (C.R. 22 cm.) brains show a vascular pattern approaching that of the adult rabbit.

Dissection of whole Monastral Fast Blue injected rabbit embryo brains shows that, in general, arteries are superficial to veins in the pial plexus, and by dissecting out individual large perforating vessels their form can be studied. The perforating veins arising in the pial plexus have a knotty form as they traverse the cortex, receiving several tributaries from the cortical arcades, which join the veins at about right angles; in contrast, the perforating arteries arising from the pial arteries pass straight down through the cortex, generally giving no branches until they reach the white matter.

Sections of the cerebral hemispheres of an injected crested newt brain show the blood supply to be derived from the meninges by simple long hairpin-shaped loops whose terminations are sometimes divided, giving a Y-shaped appearance (Pl. 1, fig. 5; Pl. 2, fig. 8).

DISCUSSION

The fundamental pattern of the blood supply to the cerebral hemispheres can be seen in a 21-day rabbit embryo brain. The cortex is supplied from the pia by vascular loops joined up to form arcades, and the white matter by long perforating cortico-medullary arteries. These perforating arteries supply only occasional branches to the deepest part of the cortex as they traverse it and then often run parallel to the

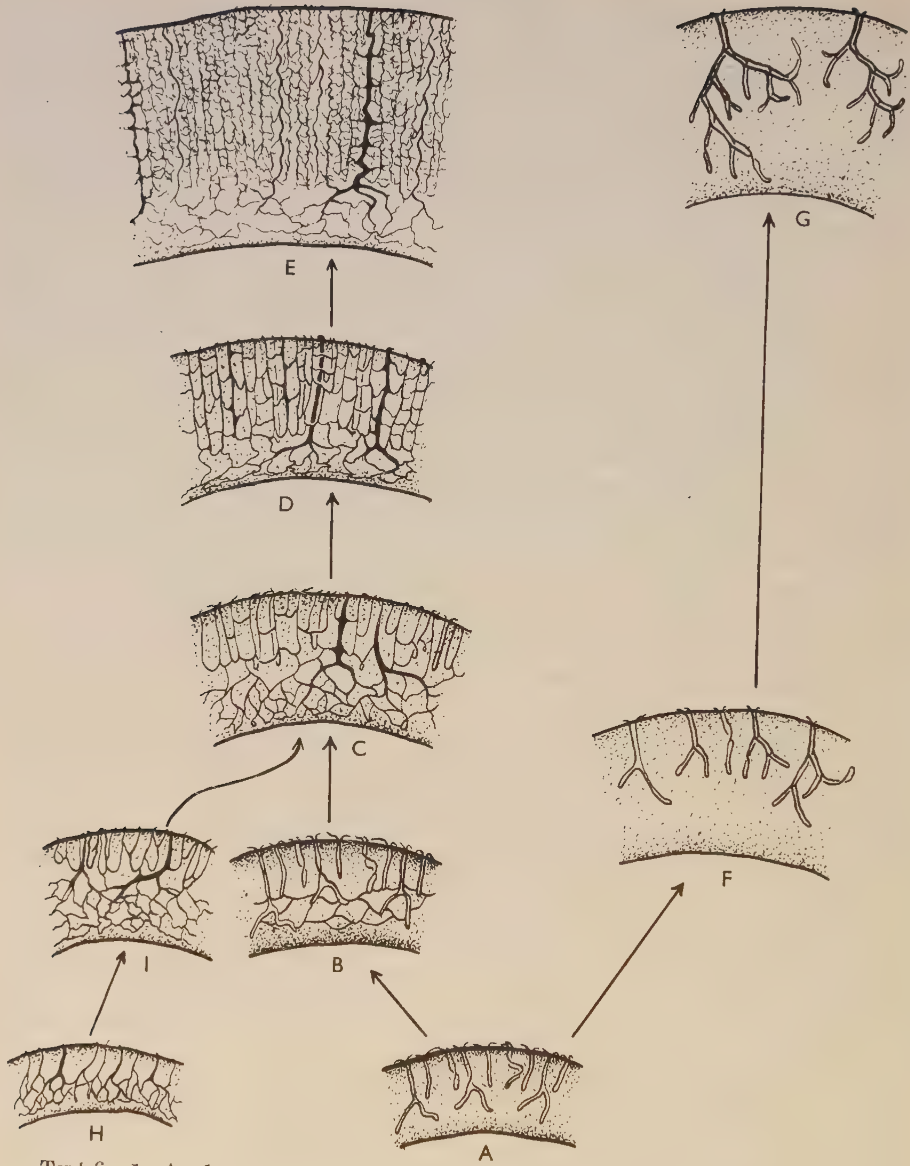
surface along the junction of cortex and medullary substance for a short distance, giving a few branches to the deep aspect of the cortical capillary bed before terminating in the white matter. There are also perforating veins, pursuing a very similar course to the arteries, but the veins receive a number of tributaries from the cortical arcade system which join them at approximately right angles and give them a rather knotty appearance. There are capillary anastomoses between cortical and medullary capillary beds, and at this stage there is little difference in the vascularity of cortex and medulla. Among the vascular arcades of the cortex are occasional hairpin loops which are strikingly similar to those seen in the newt brain.

Later there is a progressive appearance of many cross-anastomoses in the arcade system of the cortex, together with elaboration of more definite arteries and veins from some of its radial vessels, leading to a great increase in vascularity of the cortex which becomes much more vascular than the white matter.

Enlargement of those fine channels arising from the perforating cortico-medullary arteries and veins and joining the deep aspect of the cortical capillary bed leads to the appearance of large vessels approaching the deep aspect of the cortex. These would appear to be the vessels erroneously described by Pfeifer and by Cobb as deep medullary vessels, probably coming from the perforating basal vessels. In reality they are recurrent branches of the long perforating cortico-medullary arteries or veins, and thus the whole of the cortex draws its blood supply from the surface pia mater. Hence, as Sunderland (1938) found, obliteration of the surface pial vessels leads to degeneration of the whole thickness of the cortex together with a varying amount of the subjacent white matter.

There is a marked similarity between certain of these developmental stages in eutherian mammals and the pattern seen in some lower vertebrates. Thus, the vascular pattern of a 21-day rabbit embryo brain is very like that described in *Ambystoma* and in the lungfish *Protopterus* and *Epiceratodus*; any one of these species does not exactly duplicate the condition in the rabbit which rather presents a composite picture of the features of all three. This stage is also comparable to the newt brain for simple hairpin loops are occasionally present and the arcades themselves could be looked upon as derivatives of loops through the establishment of cross-connexions. It is interesting to note that Wislocki (1939) has described occasional anastomotic connexions between the simple loops of the embryo opossum brain.

Thus it may be deduced that the simple looped system is the primitive form and has been transmitted from cyclostomes to crossopterygians, amphibia, reptiles and primitive mammals. To meet the needs of an enlarging brain in any class this pattern has been elaborated into either an arborization of paired vessels (e.g. opossum, lizards) or a reticular pattern (e.g. eutherian mammals), though in some cases the elaboration into a reticular type has not been complete, giving rise to an intermediate type (e.g. *Epiceratodus*). The mode of development in higher mammalia amply supports this view. Although the very earliest stages in this hypothetical evolution of the pattern are not repeated during development in the higher mammals, a point is quickly reached which corresponds to an early phylogenetic stage and which still contains features of the most primitive pattern. This is quite in accord with the changes seen in the development of other structures.



Text-fig. 1. A scheme illustrating the development and evolution of the two types of vascular pattern.

In Text-fig. 1 the probable evolution of a reticular from the simple looped pattern of the urodele amphibian is represented by A, B, C, D, E and of the opossum type from the looped pattern by A, F, G. The actual stages of development seen in the eutherian mammal are represented by H, I, C, D, E. These developmental stages quickly approach and then coincide with the hypothetical evolutionary stages; a stage such as I is very similar to *Ambystoma* or *Epiceratodus* and even C, with its occasional hairpin loops, still has some of the features of A.

The apparently greatly different patterns in adult marsupials and eutherian mammals represent divergent elaborations of the looped system of the urodele amphibia, and their mode of development supports this view.

SUMMARY

1. In the early mammalian embryo the blood vessels of the cerebral cortex form a system of loops or arcades with larger perforating arteries and veins penetrating to the subjacent white matter and giving branches joining the deep aspect of the cortical system of vessels.

2. The perforating arteries and veins, while having a very similar distribution, do show fairly characteristic differences in form.

3. During development there is a progressive elaboration of the cortical arcade system.

4. The deep medullary vessels which have been described as supplying the deep aspect of the cortex are in reality recurrent branches of the long perforating cortico-medullary vessels.

5. A stage of development such as that of a 21-day rabbit embryo shows a pattern very similar to *Epiceratodus* and *Ambystoma* and little in advance of that of the newt.

6. Embryology provides evidence that a looped system of vascular supply is primitive and the reticular type and opossum types are derived from it.

I would like to thank Prof. D. V. Davies for advice and Messrs G. Maxwell and J. Fenton for technical assistance and photography.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. A section (400 μ) of the injected parietal lobe of a 21-day rabbit embryo brain showing cortical capillary loops and long perforating cortico-medullary arteries and veins. × 30.

Fig. 2. Another section (400 μ) of parietal lobe of same brain as fig. 1. Branches from the long perforating vessels (two veins and an artery) to the deep aspect of the cortical loop or arcade system can be seen. × 65.

Fig. 3. A section (400 μ) of the parietal lobe of the injected brain of a 26-day rabbit embryo showing a perforating cortico-medullary vein. There is here even greater elaboration of the cortical arcades than in a 23-day specimen (fig. 4). × 65.

Fig. 4. A section (400 μ) of the parietal lobe of an injected 23-day rabbit embryo brain. There is a greater elaboration of cortical arcades than in fig. 2. × 65.

Fig. 5. A section (400 μ) of the injected brain of a crested newt showing typical capillary loops penetrating from the surface. × 65.

Fig. 6. A section (400 μ) of the cerebral hemisphere of an injected 16-day rabbit embryo showing radially penetrating vessels joining a plexus in the deeper parts of the wall. × 25.

Fig. 7. Part of a section (400 μ) of the cerebral hemisphere of a 24 mm. rat embryo showing a simple loop entering from the pia mater. A branch is, however, given off from one limb of the loop. × 140.

Plate 2

Fig. 8. The same brain as fig. 5 showing both single and bifid capillary loops. × 65.

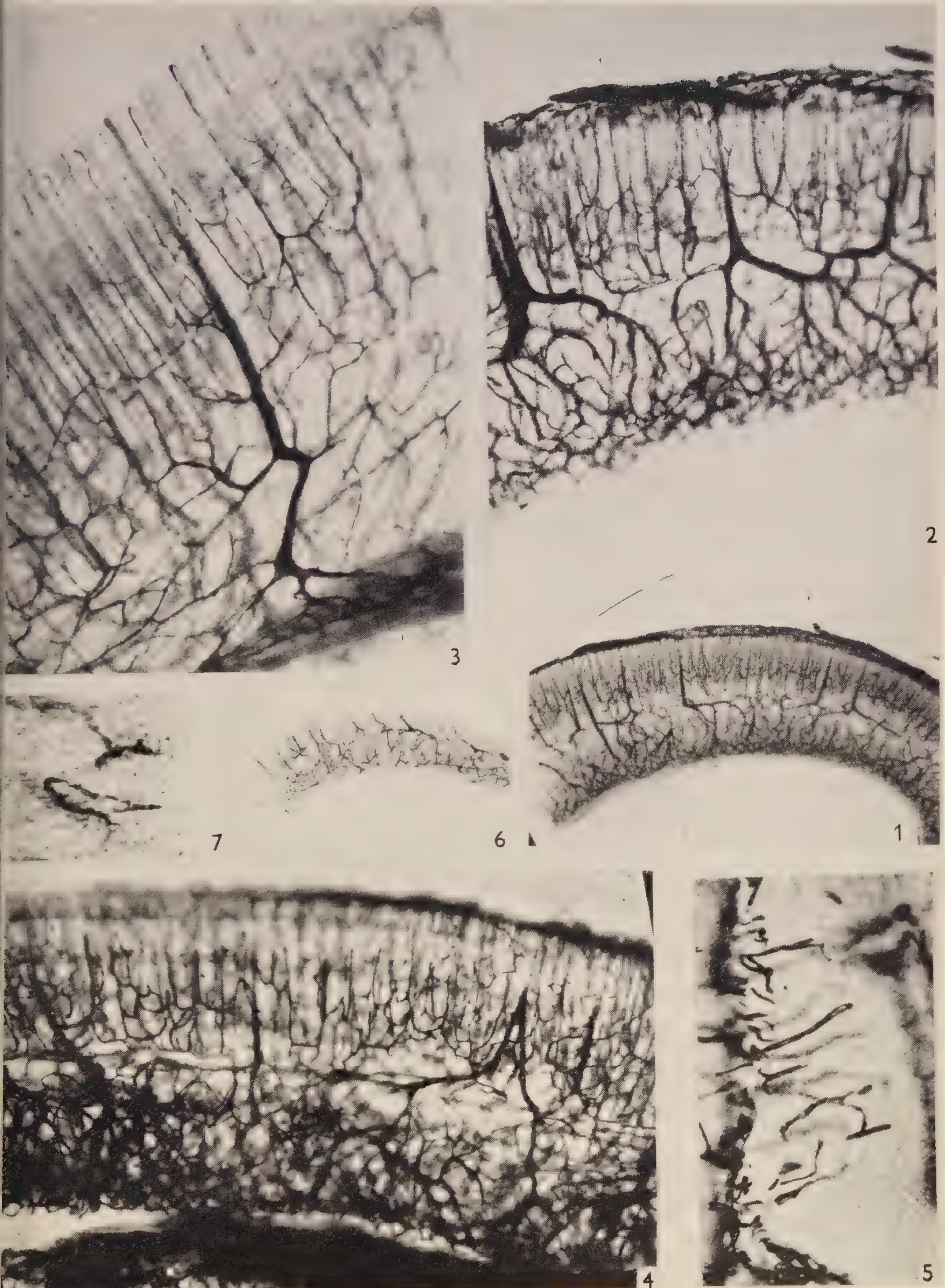
Fig. 9. A section (400 μ) of the parietal lobe of an injected adult rabbit brain showing the very vascular cortex with the less vascular white matter containing large vessels apparently approaching the cortex from below. × 32.

Fig. 10. A higher power view of the superficial layers of the cortex of the same brain as fig. 9. × 65.

Fig. 11. The junction of cortex and white matter of the same brain as fig. 9 showing the large vessels approaching and joining the deep aspect of the cortical capillary bed to be recurrent branches of the long perforating cortico-medullary vessels. In this case the perforating vessel is a vein. × 65.

Fig. 12. A section (400 μ) of the cerebral hemisphere of a 21-day rabbit embryo showing a simple hairpin loop penetrating the cortex from the pia mater. Compare with the loops in fig. 5. × 140.

Fig. 13. Another simple loop from the same brain as fig. 12. × 140.



LEWIS—BLOOD VESSELS OF THE MAMMALIAN CEREBRAL CORTEX

(*Facing p.* 46)

LEWIS—Blood vessels of the mammalian cerebral cortex

THE MENINGEAL RELATIONSHIPS OF THE GLYCOGEN BODY IN THE CHICK

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The glycogen body is a peculiar structure found near the caudal end of the avian spinal cord. It is a gelatinous mass which fills a rhomboidal fossa formed by the divergence of the posterior columns of the cord at the level of the large dorsal roots which are distributed through the sciatic nerves. The body is larger than the fossa and produces an oval swelling on the dorsal surface of the cord (Pl. 1, fig. 1). Some investigators (Streeter, 1904; Kappers, 1924) allotted primary importance to the fossa itself, terming it the 'lumbo-sacral sinus' or 'sinus rhomboidalis'. Kappers (1924) considered that the fossa was produced by the pull of the related dorsal sciatic nerve roots. Other workers (Terni, 1924; Watterson, 1947) considered that the separation of the posterior columns is a consequence of the development of the gelatinous mass. The latter workers showed that the cells of this mass contain large quantities of glycogen, as least during the last two weeks of incubation and the first three after hatching. This, the only known function of this unique mass of cells, was emphasized by these workers when they named the structure 'glycogen body'.

The relationships of the glycogen body to the meninges of the spinal cord are still a matter of controversy. Hansen-Pruss (1923) and Kappers (1924) held that the glycogen body lay between the lepto-meninges. It is difficult to reconcile their statements with the fact, which both Hansen-Pruss and Kappers accepted, that the central canal of the spinal cord passes through the glycogen body. On the other hand, Watterson (1949) supports the view expressed by Duval (1877), von Kölliker (1902) and others that the glycogen body is wholly subpial.

The conflicting views as to the position occupied by the glycogen body hinge largely on the presence or absence of a meningeal layer between the body and the tissues of the spinal cord. If such a layer exists it would argue strongly in favour of the view that the glycogen body lies in the subarachnoid space and that its presence is probably secondary to the formation of the sinus rhomboidalis. It would still be necessary to account for the unusual position of the central canal. On the other hand, a subpial position would support those investigators who assigned a primary importance to the body itself.

MATERIALS AND METHODS

Chick embryos of 8, 10, 12, 15 and 21 days incubation were used in the investigation. One full-grown male was also examined. Each embryo was decapitated after removal from the shell; the abdomen was opened and eviscerated. This procedure enabled the portion of the vertebral column containing the glycogen body to be readily identified by the position of the sciatic nerve roots. This part of the column was excised and fixed without further dissection in 5% formol-saline. The specimens were dehydrated and embedded in paraffin wax, the 21-day embryo and the adult

specimens having been decalcified in 4% nitric acid. After decalcification the vertebral column of the adult specimen was dissected away from the spinal cord and glycogen body to facilitate the preparation of sections. Sections were cut serially in the transverse plane at 10μ. For the identification of the meninges the stain employed was the modification of Long's stain used by Millen & Woollam (1954) in their study of the reticular perivascular tissue of the central nervous system. The identification, in the younger embryos, of the cells forming the glycogen body was facilitated by the use of the periodic acid-Schiff technique (Hotchkiss, 1948) to stain the glycogen granules within the cells.

OBSERVATIONS

In the 8-day embryo the glycogen body is a wedge-shaped mass of rather darkly staining cells situated in the dorsal aspect of the spinal cord. This mass separates the posterior funiculi and extends through the whole depth of the cord from its posterior surface to the large central canal. Since the tissues are only weakly argyrophilic at this stage it is difficult to be certain about the exact meningeal relationships (Pl. 1, fig. 2). The meninges appear to lie entirely dorsal to the body, i.e. the body is wholly submeningeal in position. By the 10th day the meninges are strongly argyrophilic and a layer of pia mater can be distinguished closely applied to the surface of the spinal cord. At the lateral margins of the glycogen body this layer appears to split into two laminae, one of which continues over the dorsal surface of the glycogen body whilst the other forms a small septum between the lateral part of the glycogen body and the posterior column (Pl. 1, fig. 3).

The meningeal relationships of the middle part of the glycogen body are little altered in the 12-day embryo, although the pial septum extends somewhat more deeply into the substance of the spinal cord (Pl. 1, fig. 4). The most striking alteration is to be seen at each end of the body where the pia mater now forms a complete septum which extends across the cord and separates the glycogen body from the nervous tissue (Pl. 1, fig. 5).

The size of the glycogen body increases and by the 15th day, at about its midpoint, it is beginning to surround the central canal of the cord (Pl. 1, fig. 6). Further growth takes place until, by the 21-day stage, the ventral part of the glycogen body encloses most of the related portion of the central canal (Pl. 1, fig. 7). In the later stages of incubation there is little change in the pial relationships of the glycogen body except at the cranial and caudal ends where a greater length of the body is completely separated from the spinal cord by the pial septum. In transverse sections near the ends of the glycogen body it is now possible to distinguish two parts of the body, a more dorsal part which is bounded ventrally by a pial septum and a more ventral part which surrounds the central canal and is in direct continuity with the nervous elements. The meningeal relationships in the adult specimen show that no change has occurred since the 21-day stage of incubation.

In its final form the glycogen body has a collar-stud shape. The larger dorsal portion is surrounded by the pia mater but is joined by a broad stem to the smaller ventral portion, which is not separated from the spinal cord by a meningeal layer. The central canal of the spinal cord does not at any place pierce the pial septum nor even come in close relationship with it.

DISCUSSION

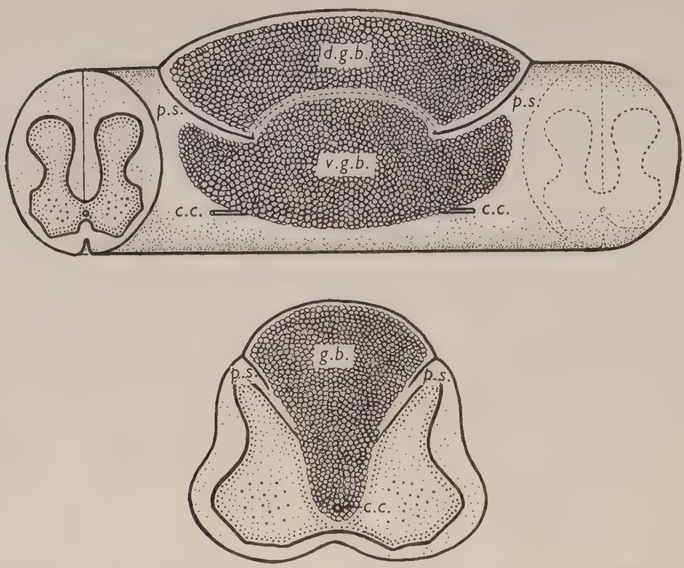
These observations on the relationships between the meninges and the glycogen body are not in complete accord with the findings of previous workers.

Hansen-Pruss (1923) and Kappers (1924) expressed the view that the glycogen body was derived from the meninges which invaded and filled the space left by the divergence of the posterior columns. They believed that a layer of pia mater lined the floor of the rhomboidal fossa and entirely separated the glycogen body from the neural tissue. Indeed Hansen-Pruss regarded the glycogen body as formed by isolated and somewhat modified subarachnoid spaces. Hansen-Pruss, however, employed routine histological stains which were not adequate to differentiate clearly the meningeal layers. Furthermore, he may have been misled by the appearance of sections near the ends of the glycogen body. In these regions, as the present investigation shows, a complete pial septum can readily be traced deep to the dorsal part of the glycogen body. However, nearer the middle of the body the pial septum extends inwards from the surface for only a short distance and the glycogen body is for the most part directly contiguous with the nervous tissue. The central canal of the spinal cord runs through the ventral portion of the body so that it is nowhere necessary for it to pierce the pia mater.

The other view that has been widely held, for example by Duval (1877), by von Kölliker (1902) and, more recently, by Watterson (1949), is that the glycogen body is derived from the cells of the neural tube and is wholly subpial.

In a series of excellent papers Watterson (1949, 1952, 1954) has provided convincing evidence of the association between the body and the cells forming the roof plate of the neural tube. The present work, whilst not particularly concerned with the question of the origin of the glycogen body, does provide indirect support for this view. Glycogen could be demonstrated in the cells of the body at the 8-day stage of incubation before there was any clear differentiation of the meningeal layers. Moreover, at no stage were the cells of the glycogen body completely enveloped by the meninges nor could any strands of meningeal tissue be seen between the cells. It does, however, appear that the meningeal relationships of the body are not as simple as they are stated to be by Watterson. The stains employed by Watterson were not sufficiently specific to demonstrate clearly the meninges. The use of a reticular stain makes it possible to see that by the 10th day of incubation a thin but definite pial septum separates the dorsal part of the glycogen body, on either side, from the spinal cord. By the 12th day the cranial and caudal ends of the body are cut off from the nervous tissue by a complete pial layer. In the fully grown fowl this unusual relationship persists so that the dorsal part of the glycogen body is embraced by the pia mater whilst the ventral portion is directly in contact with the nervous substance of the cord (Text-fig. 1). It would appear more correct to describe the glycogen body as in part intrapial and in part subpial rather than as wholly subpial.

The question how the dorsal part of the glycogen body becomes intrapial in position remains unanswered. It may be that the appearance of the pial septum at the 10-day stage is due to an active ingrowth of the pial tissue between the body and the cord or, on the other hand, the enlarging glycogen body may insinuate itself between two layers produced by a splitting of the pia mater.



Text-fig. 1. Diagram to illustrate the relationships of the pia mater to the glycogen body. *c.c.*, central canal; *g.b.*, glycogen body; *d.g.b.*, dorsal portion of glycogen body; *v.g.b.*, ventral portion of glycogen body; *p.s.*, pial septum.

SUMMARY

The development of the meningeal relationships of the glycogen body is described. In the definitive condition, the dorsal surface of the body is covered by the general pial investment of the spinal cord. At the margins of the glycogen body a pial septum passes inwards between the body and the spinal cord. The free edge of this ingrowth encircles a constricted neck of the glycogen body, between a dorsal part, separated from the spinal cord by the pial ingrowth and a ventral part continuous with the cord tissues. The central canal passes through the ventral portion. The glycogen body is, therefore, partly intrapial and partly subpial in position.

Our thanks are due to Prof. J. D. Boyd for his advice and encouragement in the preparation of this paper.

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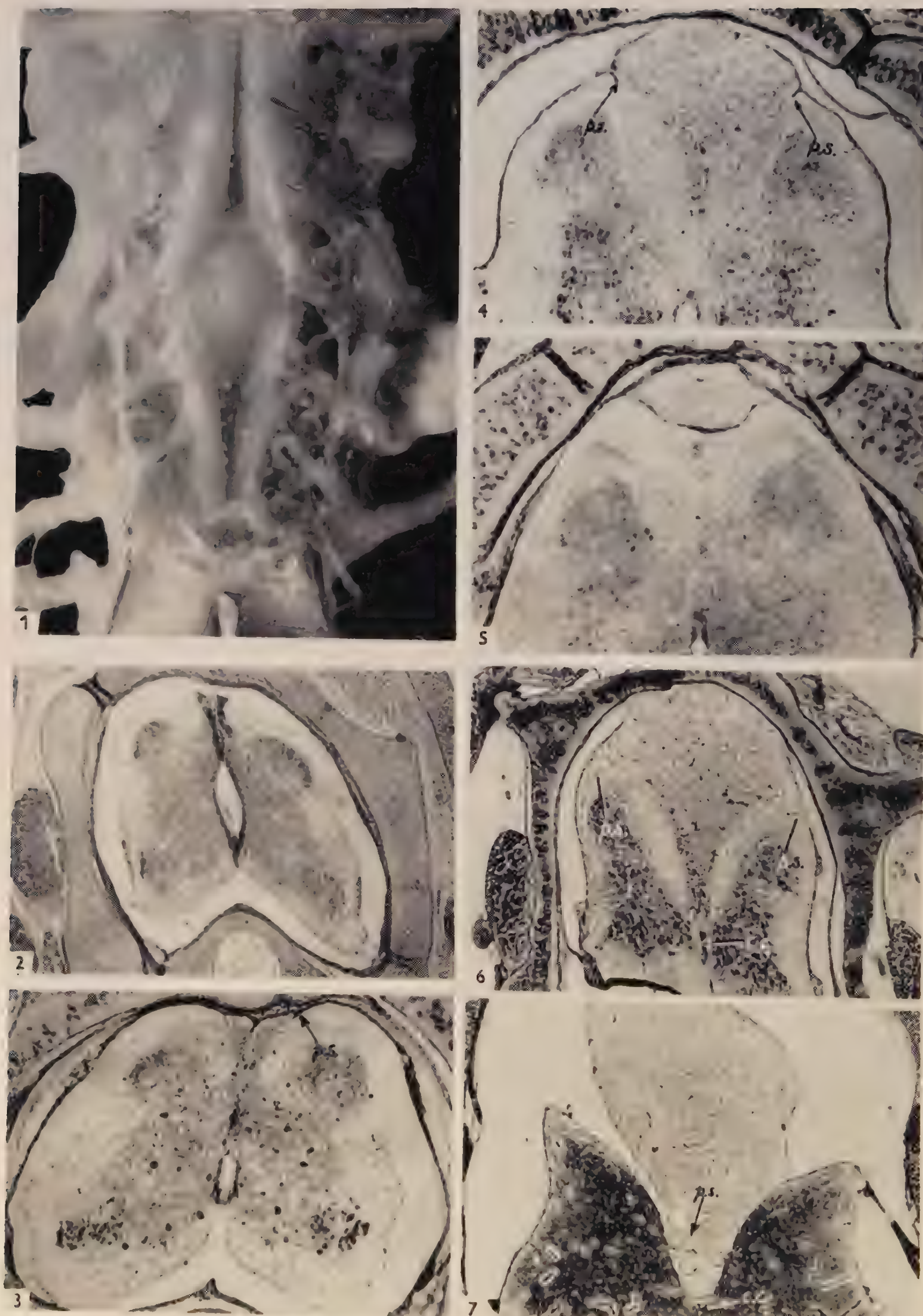

DICKSON AND MILLEN—MENINGEAL RELATIONSHIPS OF THE GLYCOGEN BODY IN THE CHICK

(*Facing p.* 51)

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EXPLANATION OF PLATE

Fig. 1. Dorsal view of spinal cord of a 22-week chicken, showing the glycogen body with the roots of the sciatic nerve on either side. (×35.)

Fig. 2. Transverse section of the spinal cord of an 8-day chick embryo. The glycogen body extends from the central canal to the dorsal surface of the cord. Reticulum stain. (×45.)

Fig. 3. Transverse section of the spinal cord of a 10-day chick embryo. The pial septum (*p.s.*) can be seen on one side of the glycogen body in this section. Reticulum stain. (×45.)

Fig. 4. Transverse section of the spinal cord of a 12-day chick embryo, passing through the middle of the glycogen body. The pial septum (*p.s.*) can be seen on each side. Reticulum stain. (×45.)

Fig. 5. Transverse section of the spinal cord of a 12-day chick embryo. The section passes through the glycogen body near its cranial end, and shows the septum which separates each end of the body from the spinal cord. Reticulum stain. (×45.)

Fig. 6. Transverse section of the spinal cord of a 15-day chick embryo. The section passes through the middle of the glycogen body, which is beginning to surround the central canal (*c.c.*), pial septum (*p.s.*). Reticulum stain. (×22.)

Fig. 7. Transverse section of the spinal cord of a 21-day chick embryo, passing through the glycogen body towards its cranial end. The central canal (*c.c.*) is surrounded by the glycogen body. The pial septum (*p.s.*), which is incomplete in this section, separates the dorsal and ventral parts of the body. Reticulum stain. (×22.)

THE CONVOLUTIONAL PATTERN OF THE BRAIN AND ENDOCRANIAL CAST IN THE FERRET (*MUSTELA FURO* L.)

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INTRODUCTION

In mammals such as the Carnivora, a cast of the cranial cavity reproduces the shape of the brain and the pattern of its convolutions (Black, 1915). In others, including man and the great apes, the endocranial cast shows very few, if indeed any, convolutional markings, particularly in the part which underlies the vault (e.g. Primates: Symington, 1915; Clark, Cooper & Zuckerman, 1936; Hirschler, 1942; Connolly, 1950. Cetacea: Breathnach, 1955). Since, in the study of fossilized skulls, the best evidence of the convolutional pattern of the brain is provided by the endocranial cast, it is of interest to establish how the correspondence (or otherwise) of the convolutional patterns of the brain and endocranial casts arises.

Theoretically there are three possible explanations: (1) that the convolutional pattern originates in the brain, which imprints it on the skull; (2) that it originates in the skull and is imprinted on the brain; and (3) that neither the convolutional pattern of the brain nor the markings on the skull are dependent on the other, but that the growth of each is separately dependent on a common factor. Only the first and the last of these explanations merit consideration. Although the first is the one commonly accepted—that the brain imprints itself on the skull—it has never been clear how the imprinting takes place as the two surfaces are separated by a layer of cerebrospinal fluid which would be expected to distribute intracranial pressure uniformly.

In the present investigation it has been possible to bring forward evidence in favour of the first of these explanations by observing whether an experimentally induced irregularity in the surface of the brain imprints itself on the inner surface of the skull.

In addition, some of the results presented here support an hypothesis which Müller (1908) put forward to explain how the imprinting takes place.

MATERIAL AND METHODS

Animals

A total of 101 albino ferrets (*Mustela furo* L.) was used. The ferret was chosen because it has a brain whose convolutional pattern is accurately reproduced on the endocranial cast. Most of the animals were males and were operated upon when they were 10–20 days old and were still being suckled by their mothers.

Operations were performed on seventy-six animals, while the remaining twenty-five animals provided normal adult material for comparison with the operated

animals. Of the latter, only twenty-one animals survived for a period adequate for the purposes of the experiment. Of these, fourteen belonged to a control group.

The age and the weight of each animal at operation and at death are given in Table 1.

Experimental procedure

The animals were anaesthetized by an intraperitoneal injection of pentobarbital (0·04 mg./g. body weight) or bromethol (0·0002 ml./g. body weight). The skin and other tissues overlying the cranial vault were incised in a parasagittal plane, and a site for trephining the skull selected between the upper margins of the temporal muscles. After reflecting the periosteum at the point selected, a hole approximately 3 mm. in diameter was bored through the bone with a dental flat-fissure burr, and the dura mater and arachnoid mater were incised parallel to any blood vessels seen within or beneath them. The exposed brain was coagulated with a diathermy knife, incised with a scalpel, and an arbitrary amount of brain tissue sucked out, as far from the trephine hole as possible, by means of a curved copper tube, 2 mm. in diameter, connected to a suction pump. In the control group of animals, the incision and suction of the brain were omitted.

Autopsy

The animals were killed with chloroform between 50 and 200 days after operation, and their tissues fixed by perfusion with 10 % formol saline. The head was removed, the skull divided in a horizontal plane close to the base, and the brain carefully lifted out of the upper part of the cranial cavity, together with its leptomeningeal coverings. Any adhesions, which occasionally occurred between the brain and the dura mater at the site of the trephine, were severed with a knife.

Preparation of casts

Plaster casts of the cranial cavity were made before and after removal of the dura mater. The cavity was cast with an alginate dental impression material which, when set, is sufficiently elastic to allow removal from the skull without damaging the bone. From the cast a replica of the cranial cavity was constructed in a brittle plaster of Paris of the type used for dental impressions. Finally, the replica was cast in a stone plaster, a dilute alginate solution being used as a separating medium. Although this method of casting involved three reproductions of surface features, there was no apparent loss of detail, the fine vascular markings of the endocranial surface nearly always being reproduced accurately. The brains and their corresponding endodural and endocranial casts were then compared.

The terms used here for the gyri and sulci of the brain of the ferret are those used for the presumably corresponding features of the brains of related carnivores (Papez, 1929; Kappers, Huber & Crosby, 1936). A reference diagram is shown in Text-fig. 1.

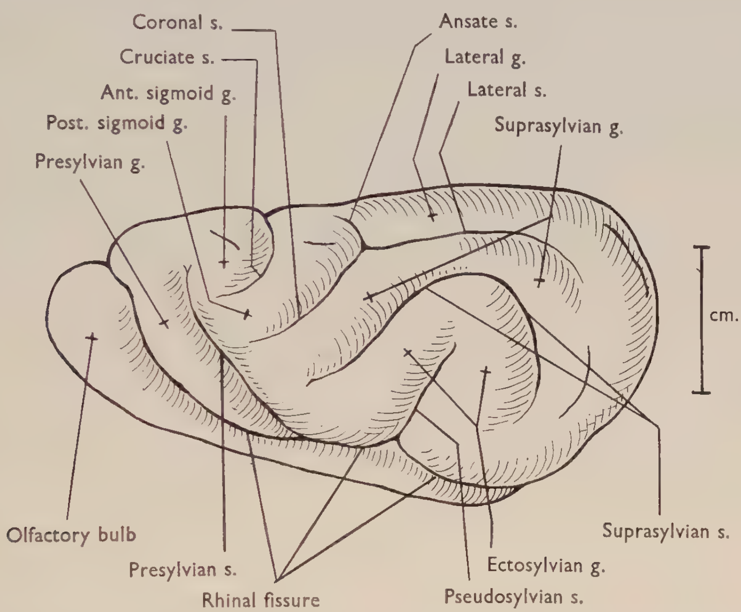
RESULTS

Abnormalities of the convolutional pattern and irregularities of the surface were found in the brains and endocasts (endodural and endocranial casts) of the ferrets from which part of the brain had been removed. Before these abnormalities could

Table 1. *Summary of the operations performed, and of the changes observed in the brains of twenty-one operated ferrets*

Animal no.	Sex	Age at operation (days)	Weight at operation (g.)	Age at death (days)	Weight at death (g.)	Type of operation	Brain changes: Meningeal adhesions at site of trephine	Surface changes	Convolutional pattern
11	M.	9	39	69	345	Experimental	?	None	Grossly asymmetric
12	M.	9	42	52	253	Experimental	?	Slight	Grossly asymmetric
107	M.	10	38	145	610	Experimental	Yes	Large depression	—
108	M.	10	40	145	840	Control	No	Slight	Slightly asymmetric
117	M.	10	44	160	1200	Control	No	Slight	Symmetric
119	M.	10	34	164	900	Control	No	Slight	Slightly asymmetric
120	M.	10	35	164	975	Control	No	Slight	Slightly asymmetric
110	F.	11	41	97	540	Control	Yes	Slight	Symmetric
109	M.	11	40	145	795	Control	No	Slight	Symmetric
112	M.	11	45	148	1195	Control	?	Slight	Slightly asymmetric
134	M.	14	75	147	1095	Control	Yes	Slight	Symmetric
100	M.	16	74	120	1110	Control	No	None	Slightly asymmetric
104	M.	18	86	120	1360	Experimental	No	Large depression	—
113	F.	18	87	145	560	Experimental	Yes	Large depression	—
101	M.	18	49	120	775	Control	No	None	Symmetric
102	M.	18	56	120	?	Control	No	Slight	Symmetric
103	M.	18	59	120	800	Control	No	None	Symmetric
123	M.	18	63	162	880	Control	Yes	Slight	Symmetric
125	M.	18	66	162	1180	Control	No	None	Symmetric
93	M.	19	94	390	1325	Experimental	Yes	Large depression	—
94	M.	*c.* 270	*c.* 930	*c.* 470	1025	Experimental	Yes	Large depression	—

be attributed unequivocally to the direct operative interference with the brain, it was necessary to know whether and how frequently similar abnormalities occurred in control-operated and normal unoperated animals.



Text-fig. 1. Lateral aspect of the forebrain of the adult ferret (*Mustela furo* L.) showing a common arrangement of the convolutions and sulci. Apart from the ansate sulcus, only the major sulci are labelled.

Normal and control-operated animals

In the twenty-five normal adult ferrets the basic convolutional pattern conformed to that shown in Text-fig. 1, though there were, however, slight individual variations which involved mainly the ansate and other minor sulci, and the junction of the lateral and coronal sulci. In eight animals minor differences also existed between the two hemispheres. Similar slight asymmetries of the convolutional pattern were likewise found in the brains of five of the fourteen animals on which control operations had been performed (see last column of Table 1). Asymmetry of the convolutional pattern was thus found in about a third of the animals of both the control-operated and normal groups.

In the control-operated animals there were, in addition, very slight superficial lesions consisting of localized thickenings of the arachnoid mater in the immediate vicinity of the site of the trephine (see last column but one of Table 1).

The endodural and endocranial casts of the control-operated animals faithfully reproduced the convolutional patterns of the corresponding brains, and only differed from one another with respect to vascular markings and surface texture.

Experimental animals

Brains

The operated hemispheres of the brains of all the experimental animals (nos. 11, 12, 93, 94, 104, 107 and 113) showed distinct abnormalities, while the unoperated hemispheres were normal, being indistinguishable from hemispheres of the normal and control-operated animals. On the whole, two types of abnormality could be distinguished. In one group, comprising animals 11 and 12, the convolutional pattern was altered; in the other (animals 93, 94, 104, 107 and 113) there were depressions in the cerebral surface, the convolutional pattern being otherwise unaltered.

The convolutional patterns of the brains of animals 11 and 12 were abnormal though well defined (Pl. 1, figs. 1, 3). In animal 11 the cruciate and presylvian sulci and the anterior end of the rhinal fissure were conjoined, the presylvian gyrus was correspondingly reduced in size, and the junction of the anterior and posterior sigmoid gyri was absent. In animal 12 there was a localized thickening of arachnoid mater lying immediately under the site of the trephine at the lateral end of the cruciate sulcus, and towards this point there converged the cruciate, coronal, suprasylvian and presylvian sulci, the minor sulcus normally lying anterior to the cruciate, and an abnormal sulcus crossing the presylvian gyrus. The usual junction of the anterior and posterior limbs of the sigmoid gyrus was absent.

In contrast to animals 11 and 12, the operated hemispheres of the remaining five animals, 93, 94, 104, 107 and 113, all presented irregular depressions in the cerebral surface varying from 1 to 3 mm. in depth (Plate 1, figs. 5, 7; Pl. 2, figs. 9, 11 and 13). In animal 107 and, to a lesser degree, in animals 94 and 113 the excavated area extended well beyond the site of the trephine. In the case of animal 94 the depression was covered by a sheet of thickened arachnoid mater which was firmly attached to the margins of the depression. In the photograph (Pl. 1, fig. 7) the margins of the depression can be seen showing faintly through this sheet. The sheet itself was torn at the site of the trephine when the brain was removed from the skull. Fragmented remnants of similar but thinner sheets of arachnoid mater were found spanning the depressions in the brains of animals 93, 104, 107 and 113. In animal 107 quite an extensive sheet of arachnoid mater covered the shallow posterior part of the cerebral depression whose upper margin is consequently obscured in the photograph (Pl 2, fig. 11). The free anterior edge of this sheet appears as a sharp posterior margin to the deeper part of the cavity in the brain.

The difference in outcome between the operations performed on animals 11 and 12 and those performed on the remaining five animals is probably due to the lesions in animals 11 and 12 being smaller than those in the other animals and not extending so deeply towards the germinal zone of the pallium. The operations on these two animals were the earliest in the series and the surgical procedures were less bold than in later animals. Another possibility, however, is that reorganization of the tissues of the ferret brain may take place if the animal is young enough at operation. The major sulci of the ferret brain appear between 6 and 8 days of age, and animals 11 and 12 were 9 days old at operation.

Endocasts

The endodural and endocranial casts of all seven experimental animals reproduced the convolutional pattern of their corresponding brains everywhere on both operated and unoperated hemispheres, except where the depressions on the cerebral surface occurred in animals 93, 94, 104, 107 and 113. In these animals, instead of depressions, the endocranial casts presented elevations whose margins mostly corresponded with those of the cerebral depressions (Pl. 1, figs. 6, 8; Pl. 2, figs. 10, 12 and 14). This is most clearly seen in the endocranial cast of animal 107 (Pl. 2, fig. 12). In animal 94 the contours of the endocranial cast conformed to those of the thickened sheet of arachnoid mater covering the cavity in the brain, the cast only presenting a small elevation at the site of the trephine (Pl. 1, fig. 8).

The endocranial cast of animal 12 exhibited a similar slight elevation at the site of the trephine, but otherwise this cast and that of animal 11 reproduced the abnormal sulcal patterns of their corresponding brains (Pl. 1, figs. 2, 4). The latter cast was the only one which presented shallow and ill-defined sulcal markings. The markings of these two endocranial casts, in particular, do not show as clearly in the photographs as they do on direct examination.

The endodural casts did not provide any useful information additional to that supplied by the endocranial casts and are not described.

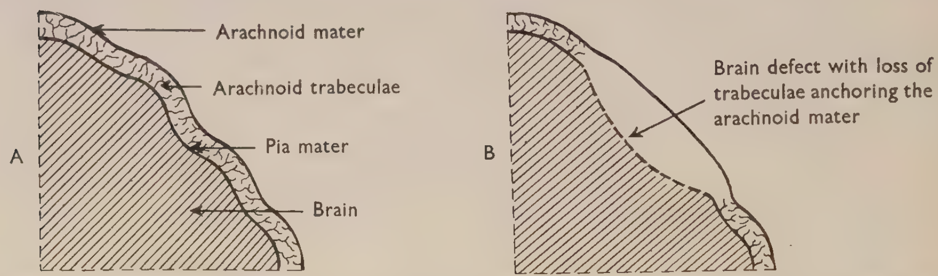
DISCUSSION

The evidence presented here suggests that the convolutional pattern of the endocranial cast in the ferret depends on that of the corresponding brain; for, whatever subsequent interactions there may have been between the brain, the meninges and the skull, a morphological change occurring initially in the brain was followed by a change in the shape of the overlying endocranial surface. The possibility that the abnormal convolutional patterns and depressions of the cerebral surface found in the experimental animals might have resulted from the trephination and division of the dura and arachnoid mater, or that it might have been present before operation, was excluded by the absence in control-operated animals of any lesions more severe than a localized thickening of arachnoid mater, and by the failure to find comparable abnormalities of convolutional pattern among control-operated and unoperated animals.

Although the results observed in the five animals presenting depressions of the cerebral surface (nos. 93, 94, 104, 107 and 113) support the above conclusion, they also show that interference with the brain and meninges may disturb the close conformity between the cerebral and endocranial surfaces which normally exists in the ferret. In those animals where there were depressions in the cerebral surface the endocranial casts presented elevations, which indicated that the cranial wall had not become thickened to occupy the space left after removal of brain tissue, but that, on the contrary, it had become thinner.

Müller (1908) has advanced an hypothesis, illustrated in Text-fig. 2, which could account for these observations. When a fresh human brain with the arachnoid mater in position is supported in such a way that liquid is allowed to distend the subarachnoid space, the surface of the arachnoid mater is thrown into folds approximately

corresponding to the convolutions of the underlying brain. This folding, he suggested, was due to the arachnoid trabeculae—connective tissue fibres binding the arachnoid mater to the pia mater. Since the dura mater is only separated from the arachnoid mater by the minute subdural space, the dura mater and bone are, he contended, bound to follow the relief of the arachnoid surface. Text-fig. 2 also shows what might be presumed to occur when brain tissue is removed and the overlying trabeculae consequently severed, as in the ferrets reported upon in this paper. The resulting depression in the endocranial surface, or elevation on the endocranial cast, would



Text-fig. 2. Schematic diagrams of a coronal section through a brain and its leptomeningeal coverings to illustrate the hypothesis referred to in the text (p. 57). A, in an intact animal; B, in an 'operated' animal.

then be due to the unrestricted expansion of the arachnoid capsule by the pressure of the cerebrospinal fluid. The thin sheet of arachnoid mater shown spanning the depression in Text-fig. 2 could be clearly seen in the brains of animals 93 and 107 when they were immersed in liquid. Fragments of such sheets were found in animals 104 and 113. In animal 94 there was a very much thicker sheet which covered the depression; this may explain why the bulge on the endocranial cast was less pronounced in this animal than in the other animals with depressions of the cerebral surface. To substantiate Müller's hypothesis it would clearly be necessary to obtain a similar result from the cutting of arachnoid trabeculae alone without incurring damage to the brain.

Müller's hypothesis requires further development if it is to explain the unequal distribution of the pressure exerted by the cranial contents on the endocranial surface which, presumably, is the immediate cause of the convolutional impressions on the surface of the bone. In the early stages of the development of a gyrencephalic mammal the surface of the brain is smooth, as are the surfaces of the surrounding capsules formed by the meninges and developing skull. As development proceeds, differing rates of expansion of various parts of the cerebral hemispheres result in the appearance of convolutions and sulci. These changes are reproduced on the surface of the capsule of arachnoid mater through the agency of the pressure of the cerebrospinal fluid and the tension of the arachnoid trabeculae connecting the arachnoid to the pia mater. Consequently, the rate at which the arachnoid capsule expands and, therefore, the pressure which it exerts on the surrounding bone and endosteum, are greater in regions overlying developing convolutions than in regions overlying developing sulci, where its expansion is retarded by the arachnoid trabeculae tethering it to the more slowly expanding sulcal areas of the cerebral surface.

Thus the resulting unequal distribution of the pressure exerted on the dura mater and endosteum derives from the differential growth of the brain. This explanation of the moulding of the endocranial surface still remains plausible even if pressure were uniformly distributed throughout the cerebrospinal fluid, provided the pressure of the fluid in the subarachnoid space puts the arachnoid trabeculae under sufficient tension to allow the reproduction of the cerebral contours on the arachnoid surface.

Barron (1950) found that destruction of substantial portions of one hemisphere of the foetal sheep's brain did not appear to alter the basic convolutional pattern of the remaining fragments, although the space available within the skull for expansion of the brain was thereby considerably increased. From these observations he concluded that the folding of the cerebral surface is due to mechanisms within the brain, and is unlikely to be initiated by any restriction of brain growth imposed by the skull envelope. As far as they go, the present findings support Barron's conclusion; for in each of the five animals in which cerebral defects persisted, the convolutional pattern of the opposite hemisphere was normal. So, too, was the remaining cortex of the operated hemisphere surrounding the depressions in the brains of animals numbered 93, 94, 104 and 113.

SUMMARY

1. The skulls of young ferrets were trephined and, in some of them, part of the brain was sucked out. Fifty days or more after operation the animals were killed and their brains compared with casts of the corresponding cranial cavities.

2. The changes in shape resulting from operative interference with the brain were partially reflected in the endocranial casts.

3. It is concluded that the convolutional pattern of the endocranial cast in the ferret is dependent on that of the brain.

4. Artificially produced depressions in the surface of the brain were reproduced as elevations on the endocranial casts. This is explained by Müller's hypothesis that the endocranial surface is moulded by the surface of the arachnoid mater, which generally duplicates the contours of the brain through the agency of the arachnoid trabeculae and the pressure of the cerebrospinal fluid, but may not do so when the trabeculae are severed.

I wish to thank Prof. Sir Solly Zuckerman and Dr J. T. Eayrs for valuable criticism and advice, and Messrs Aspin, Harcourt and Webster of the Birmingham School of Dental Surgery for instructing me in the preparation of casts.

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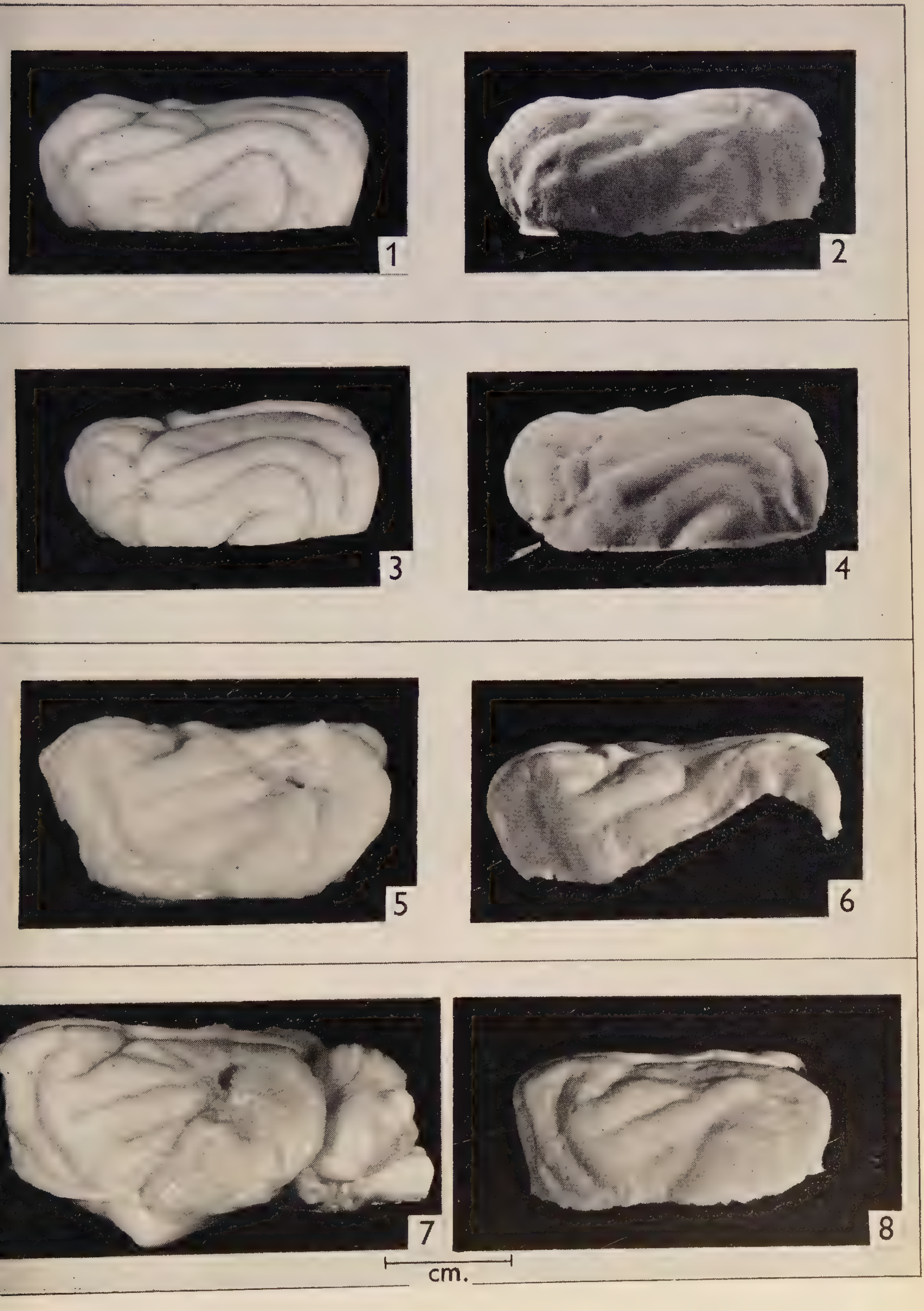
EXPLANATION OF PLATES

Plate 1

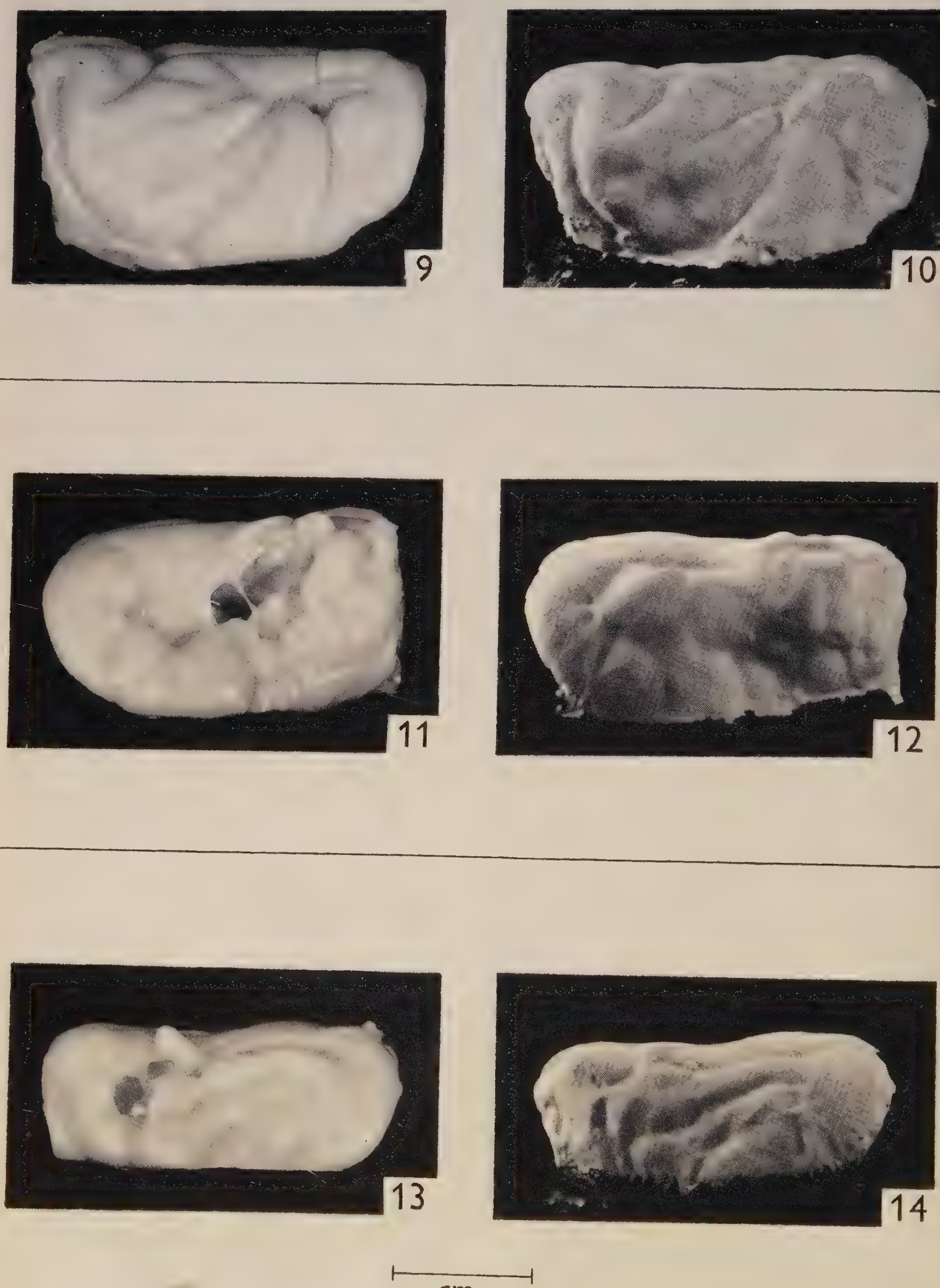
Fig. 1. Left cerebral hemisphere of animal no. 11.
Fig. 2. Left half of endocranial cast of animal no. 11.
Fig. 3. Left cerebral hemisphere of animal no. 12.
Fig. 4. Left half of endocranial cast of animal no. 12.
Fig. 5. Left cerebral hemisphere of animal no. 93.
Fig. 6. Left half of endocranial cast of animal no. 93. The area which has been blacked-out corresponds to a part of the skull which was damaged during removal of the brain post mortem.
Fig. 7. Left cerebral hemisphere of animal no. 94.
Fig. 8. Left half of endocranial cast of animal no. 94.

Plate 2

Fig. 9. Left cerebral hemisphere of animal no. 104. The fissure running in the coronal plane from the excavated area towards the mid-sagittal plane is an artifact which was produced when the brain was removed from the skull post mortem.
Fig. 10. Left half of endocranial cast of animal no. 104.
Fig. 11. Right cerebral hemisphere of animal no. 107.
Fig. 12. Right half of endocranial cast of animal no. 107.
Fig. 13. Left cerebral hemisphere of animal no. 113.
Fig. 14. Left half of endocranial cast of animal no. 113.



DARLINGTON—ENDOCRANIAL CASTS IN FERRETS



DARLINGTON—ENDOCRANIAL CASTS IN FERRETS

THE VASCULARIZATION OF THE RABBIT FEMUR AND TIBIOFIBULA

By M. BROOKES and R. G. HARRISON
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Previous investigations of the vascularization of bone have largely centred on the respective contributions of arterial blood to long bones derived from the periosteal network, the principal nutrient artery and arteries entering a bone at its extremities. The older authors paid much attention to the periosteal network (e.g., Barkow 1868; Langer, 1876), and Testut (1880) gave it primary functional significance. In more recent times this has been disputed. On the basis of injection experiments made as a preliminary to perfusion studies, Drinker, Drinker & Lund (1922) thought fit to neglect it altogether, while Johnson (1927) granted it only subsidiary importance. Their views are supported by Harris (1933), Kistler (1934), Wood-Jones (1946), Marneffe (1951), Watson-Jones (1952) and Laing (1953) who all give the principal nutrient artery a pre-eminent place in the schema of bone vascularization. Gray (1954), Testut & Latarjet (1948), Cunningham (1951) and Ham (1932) allow a free anastomosis between the terminals of this artery and the periosteal system, while only rarely is it stated to be solely of haemopoietic function (Houang, 1934).

The arteries entering the extremities of a long bone (Hunter, 1743) are generally given a supporting role in bone nutrition. With the exception of arteries entering the human femoral head and neck, there are few works in which these arteries are the subject of exact anatomical description (Marneffe, 1951; Fracassi, 1954).

If great emphasis has been placed on the arterial supply of bone, venous drainage has attracted little attention. Langer (1876) first pointed out that there are more veins leaving bone than entering arterial twigs. Lamas, Amado & da Costa (1946) have remarked upon the large 'venous lakes' existing in the cancellous bone at the extremities of long bone, while Marneffe (1951) describes a central venous channel extending the length of the medulla. In this connexion it is to be recalled that both Rustizky (1872) and Bizzozero (1869) observed a central medullary vein, the former in frogs, the latter in a rabbit tibia.

In view of the inadequacy of accounts of bone vascularization, of the rabbit in particular (Krause, 1884), it was decided to undertake an investigation of the blood supply of the rabbit femur and tibiofibula.

MATERIALS AND METHODS

Twenty-one fully grown rabbits were used, average weight 3 kg. Each was sacrificed by intravenous injection of Nembutal into an ear vein, and injected intravascularly with varying dilutions of Micropaque (Damancy and Company Ltd.), a 50% suspension of barium sulphate. After fixation in 10% formol saline solution, Micropaque is quite hard and does not run. Because of its white colour, the part could easily be dissected with the aid of a binocular microscope to determine every

vessel that pierced bone, its source, and site of penetration. Other specimens were injected with thorotrast and fixed in 70% alcohol.

The arterial system of the hind-limb was filled via the abdominal aorta; venous filling was effected through the external iliac, lateral circumflex femoral, and long saphenous veins. A polythene cannula was used for arterial injection, but hypodermic needles are more suitable for veins, since they can be used to pierce the venous valves obstructing the retrograde pathway.

Following dissection, the individual bones were stripped of all extra-osseous tissue including periosteum (Delkeskamp, 1915; Trueta & Harrison, 1953), to permit clear visualization of intra-osseous vessels alone, and to avoid confusion arising from the superimposition of vessels in soft tissues. This procedure did not remove periosteal arterial twigs from the cortex since, as will be shown later, minute vessels leaving the cortex to enter the periosteum are still demonstrable in profusion. Decalcification for 5 days in a mixture of 5% formol saline and 5% nitric acid followed. The bone was then radiographed on Kodaline film, and selected specimens were subjected to microradiography using Kodak maximum resolution plates, and a microfocus radiographic unit incorporating the Ehrenberg and Spear tube.

GROSS OBSERVATIONS

Femur: arterial supply (Text-figs. 1 and 2)

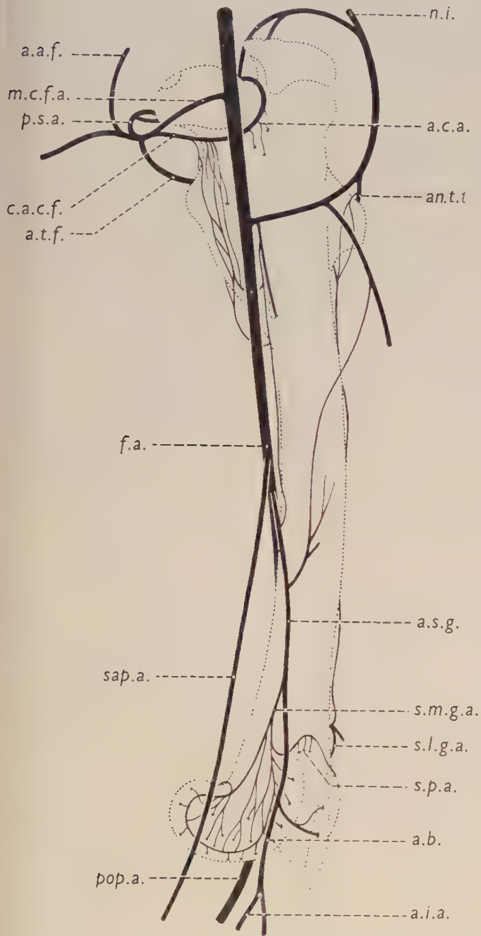
The medial circumflex femoral artery has a branch which passes laterally on the hip joint to terminate as a contribution to the trochanteric anastomosis. From it, anterior cervical arteries descend into the femoral neck. Another branch of the same artery concerned in the nutrition of the femur is the artery of the trochanteric fossa which passes downwards and laterally behind the neck to sink into a foramen in the depths of the fossa. It gives off ascending branches associated with the posterior aspects of the lesser trochanter and femoral neck, and the greater trochanter respectively, which thereby receive nutrient twigs. There are no nutrient arteries serving the third trochanter.

The posterior subcapital artery arises from the artery of the trochanteric fossa or directly from the medial circumflex femoral, and sinks into a foramen just below the rim of the head. From it posterior cervical arteries descend into the neck.

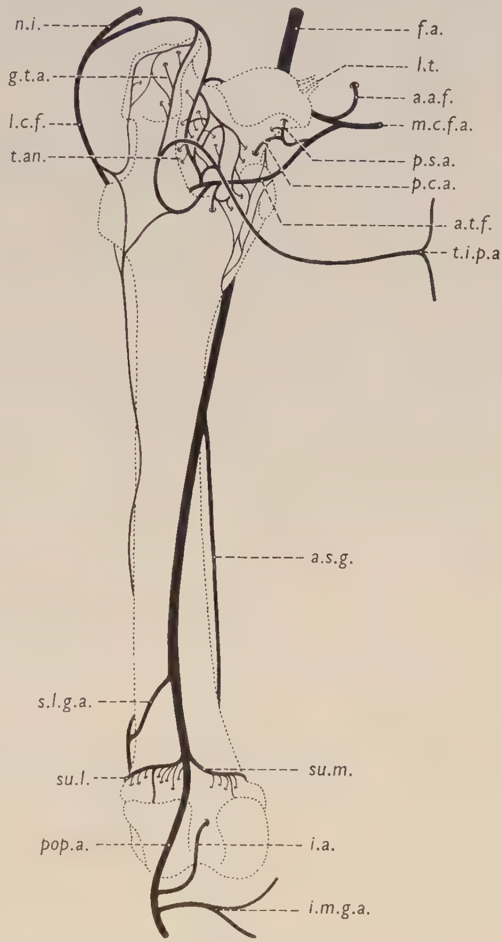
Besides anterior and posterior cervical arteries the neck of the femur is pierced by numerous small vessels derived from the anastomosis in the trochanteric fossa. This anastomosis is formed by branches of the medial and lateral circumflex femoral arteries and a vessel of small calibre which runs on obturator internus, the trochanteric branch of the internal pudendal artery.

The principal nutrient artery of the rabbit femur springs from the root of the lateral circumflex femoral artery. In one specimen it sprang directly from the femoral, distal to the lateral circumflex femoral artery. It passes downwards for 2 cm. before disappearing in the nutrient foramen situated on the medial surface of the shaft just below the lesser trochanter. It has a branch which provides twigs to the periosteum of the lesser trochanter and the substance of pectineus, and continues downwards in close association with the insertions of add. brevis, longus et magnus. It anastomoses below with the A. suprema genu.

The A. suprema genu divides into articular and muscular branches. The former descends medial to the lower end of the femur. It gives off a superior medial genicular artery which forms a loop running round the periphery of the medial aspect of the inferior extremity of the bone. Anastomotic channels traverse the area enclosed



Text-fig. 1. Drawing of arterial supply of the rabbit femur; anterior aspect. The following key applies to Text-figs. 1 and 2:



Text-fig. 2. Drawing of arterial supply of the rabbit femur; posterior aspect.

a.a.f. artery to acetabular fossa
a.b. articular branch
a.c.a. anterior cervical arteries
a.i.a. anterior intercondylar artery
a.s.g. arteria suprema genu
a.t.f. artery to trochanteric fossa
an.t.t. anastomosis round third trochanter
c.a.c.f. circulus arteriosus capitis femoris
f.a. femoral artery
g.t.a. arteries to greater trochanter
i.a. intercondylar artery
i.m.g.a. inferior medial genicular artery
l.c.f. lateral circumflex femoral artery
l.t. ligamentum teres
m.c.f.a. medial circumflex femoral artery
n.i. nutrient to ilium
p.c.a. posterior cervical arteries
pop.a. popliteal artery
p.s.a. posterior subcapital artery
sap.a. saphenous artery
s.l.g.a. superior lateral genicular artery
s.m.g.a. superior medial genicular artery
s.p.a. suprapatellar arteries
su.l. lateral supracondylar artery
su.m. medial supracondylar artery
t.an. trochanteric anastomosis
t.i.p.a. trochanteric branch of internal pudendal artery.

by the loop. From this plexiform arrangement nutrient twigs are given off which are radially disposed in relation to the circle whose centre is the medial epicondyle of the femur. Anteriorly, on the suprapatellar surface of the femur where descending nutrients pierce the anterior surface of the metaphysis, the medial condylar loop joins its fellow from the other side. This lateral condylar loop is formed by the condylar branch of the superior lateral genicular artery, which springs from the femoral about 1 cm. above the condyles, and divides into muscular and condylar branches.

The popliteal artery gives origin to medial and lateral supracondylar arteries, which pass outwards supplying fine nutrient twigs to the posterior face of the inferior metaphysis, and to the condyles. They join the medial and lateral condylar loops. From the popliteal or anterior tibial artery, a large middle genicular artery arises, which pierces the joint capsule, passes above the point of crossing of the cruciate ligaments, and sinks into a foramen in the anterior wall of the intercondylar notch.

Femur: Venous drainage

A single vena comitans accompanies each artery. At the surface of the bone at either end, the nutrient venous radicles are more numerous than the arterial, and some occupy their own canals, not sharing the space with an incoming artery. A simple circulus venosus is formed on the superficial surface of each condyle. The femoral intercondylar vein joins the tibial intercondylar veins to drain into the anterior tibial vein.

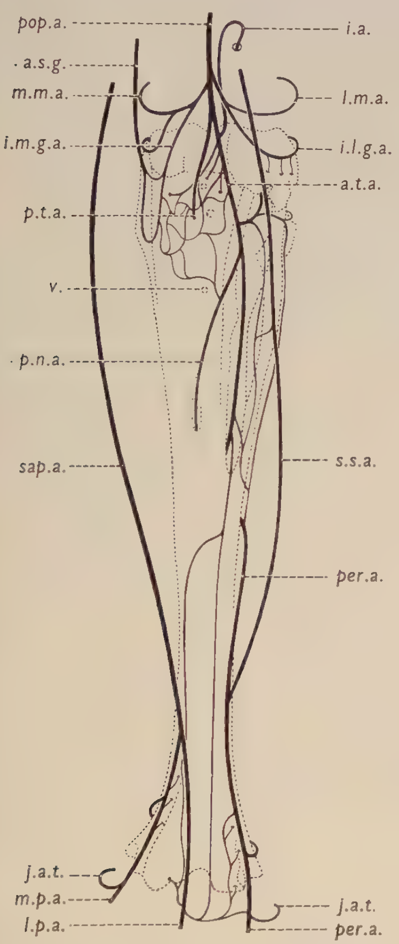
Tibiofibula: arterial supply (Text-figs. 3 and 4)

The superior tibial epiphysis is pierced in the prespinal portion of the intercondylar ridge by two anterior tibial intercondylar arteries, derived from the articular branch of the A. suprema genu. The sides of the superior epiphysis are pierced directly by nutrients derived from the medial and lateral inferior genicular arteries. The latter sends a few descending twigs into the superficial surface of the superior fibular epiphysis which is synostosed to the tibia.

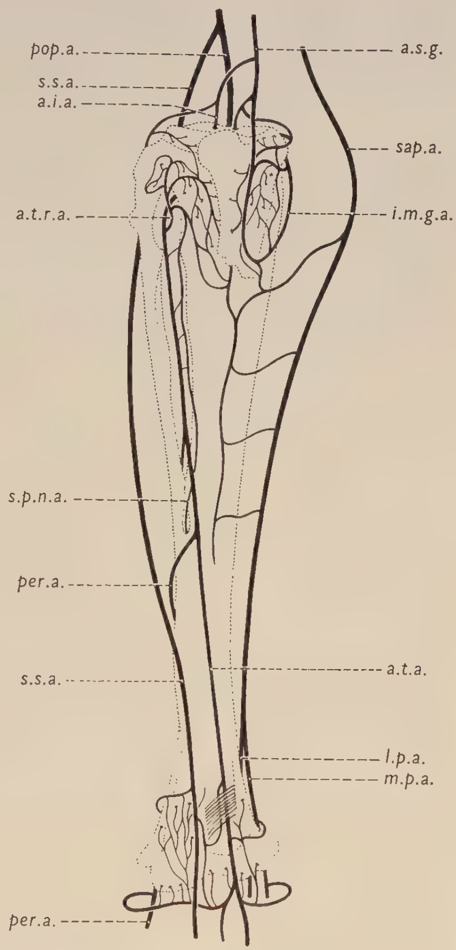
On the medial and lateral surfaces of the upper tibial metaphysis, a rich periosteal network is formed from branches of the articular limb of the A. suprema genu, the inferior medial genicular and anterior tibial recurrent arteries. From it descending nutrients pierce the cortex while some travel anteriorly to sink into the tibial tuberosity. It is linked with transverse periosteal branches of the saphenous artery, four in number, which form a longitudinal vessel related to the anterior border of the tibiofibula.

On the posterior aspect of the upper metaphysis, many nutrients pierce the bone. Some are derived directly from the anterior tibial artery, while others come off a periosteal network formed by branches of the anterior tibial and inferior medial genicular arteries. The principal nutrient artery of the tibia, derived from the anterior tibial artery, contributes to this network. It descends on the posterior surface of the bone before reaching the nutrient canal situated 5 mm. above the level of the tibiofibular synostosis. A second principal nutrient artery given off by the anterior tibial at the synostosis sinks into the bone anteriorly just below the level of fusion. The shaft of the fibular portion of the bone has no nutrient artery of its own.

The anterior tibial artery gives off constantly a periosteal vessel which descends on the posterior surface of the bone, and divides into two branches which pass downwards and take part in the anastomosis round the ankle joint. Other arteries helping to form this structure are the anterior tibial artery, its peroneal branch, and the medial and lateral plantar divisions of the saphenous artery. From it, ascending nutrient arteries pierce the inferior epiphysis all round its rim, while others pierce the metaphysis.



Text-fig. 3. Drawing of arterial supply of the rabbit tibiofibula; anterior aspect. The following key applies to Text-figs. 3 and 4:



Text-fig. 4. Drawing of the arterial supply of the rabbit tibiofibula; posterior aspect.

a.i.a. anterior intercondylar artery
a.s.g. arteria suprema genu
a.t.a. anterior tibial artery
a.t.r.a. anterior tibial recurrent artery
i.a. intercondylar artery
i.l.g.a. inferior lateral genicular artery
i.m.g.a. inferior medial genicular artery
j.a.t. joins with anterior tibial artery
l.m.a. lateral menisceal artery
l.p.a. lateral plantar artery
m.m.a. medial menisceal artery
m.p.a. medial plantar artery
per.a. peroneal artery
p.n.a. principal nutrient artery
pop.a. popliteal artery
p.t.a. posterior tibial artery
sap. a. saphenous artery
s.p.n.a. secondary principal nutrient artery
s.s.a. superficial sural artery
v. opening for emissary venous sinus

Tibiofibula: venous drainage

The veins of the tibiofibula show those general features which have been described for the femur. The following special points are to be noted.

A vein issues from the fibular border of the shaft below the synostosis and drains into the peroneal vein. On the posterior surface of the tibia, two and occasionally three large veins issue from the bone. One is found at the level of the lowest point of the tibial tuberosity, the others are subcondylar in position; all drain into the anterior tibial vein. Veins from the intercondylar ridge join those draining the inferior femoral epiphysis, and pass ultimately into the anterior tibial vein.

RADIOLOGICAL OBSERVATIONS

Arteriography

In specimens injected with undiluted Micropaque at 100 mm. Hg the following features may be discerned.

In the femur (Pl. 1, fig. 1) the principal nutrient artery traverses the cortex inclined *towards* the knee. No branches are given to the cortex in the nutrient canal. On entering the medulla it divides into ascending and descending limbs which with but few subdivisions pass to either end of the bone. Arteries in the head, greater trochanter, lower metaphysis and epiphysis, under the conditions of demonstration, appear to be discrete. In the tibiofibula there are similar arteries in the upper and lower epiphyses; the principal nutrient artery either divides into ascending and descending limbs, in which case the secondary diaphyseal nutrient is a single descending artery, or the descending limb of the principal nutrient artery may fail, when the secondary diaphyseal nutrient divides into two main descending arterial channels. In the neighbourhood of the nutrient canal of a long bone, a few fine vessels arise from the branches of the principal arteries and pass outwards into the medulla in the immediate vicinity. In general, larger arteries are visualized as sharply defined and tortuous channels disposed in the proximo-distal axis, and are comparatively few.

When a 75% suspension of Micropaque in isotonic saline is used as the injection mass more complete arterial filling can be achieved. Medullary arteries can then be traced to the metaphyseal region where they break up into numerous fine vessels which join across the line of union at the epiphyseo-metaphyseal synostosis with others derived from the epiphyseal arteries. Pl. 1, fig. 3, has been produced by injecting such an injection mass to bursting point (250–300 mm. Hg). Distinct from the oval shadows caused by drops of burst Micropaque are hazy shadows lying in the sites of epiphyseal union with the metaphysis. These are undoubtedly due to rupture at the synostosis of the medullary terminal arborizations described above. These preparations also show (Pl. 1, fig. 4) arterial twigs derived from the main medullary arteries, which pass more or less transversely towards the endosteal aspect of the compactum where they recurve and course for a short distance in the peripheral medullary zone.

The arteries of the whole bone are demonstrably in continuity with one another, epiphyseal arteries anastomosing with those supplying the diaphysis. It is therefore unlikely that the nutrient arteries at the metaphyses are end-arteries, as claimed by

Harris (1933). Although vascular ruptures have been produced by maximum filling, no arterial channels are visualized in the cortex.

In bone injected with a 50 % suspension of Micropaque in isotonic saline, at 100 mm. Hg, it is possible to produce complete filling of the arterial system of bone without the undue occurrence of vascular ruptures. Pl. 1, fig. 2, shows the extent of arterial permeation of the rabbit femur. The longitudinal disposition of the larger arteries and the transverse arrangement of the smaller ones is apparent. A medullary anastomosis exists between the artery of the trochanteric fossa and the ascending limb of the principal nutrient artery. In both femur and tibiofibula cortical filling has occurred.

Transverse sections (Pl. 1, figs. 5, 6) through the shaft show how the transverse smaller arteries pass to the peripheral medullary zone where they anastomose with one another and give rise to fine vessels which pierce the endosteal face of the compactum and arborize irregularly in the inner cortical zone. Only an occasional artery traverses the full thickness of the cortex to unite the medullary with the periosteal arterial system. The outer cortical zone has no periosteal arterial supply, and in transverse microradiography appears comparatively bloodless.

Venography

The venous pattern visualized radiographically in the whole bone shows striking differences from those arterial features described in the previous section. Using 75 % Micropaque in isotonic saline, a solitary longitudinal channel of wide calibre in an approximately central medullary position can be traced from one end of the bone to the other. In the femur (Pl. 2, fig. 1) this channel passes from the trochanteric fossa downwards, is joined by the principal nutrient vein a short distance below the nutrient canal, and passes characteristically as a single vessel down to the inferior metaphysis where it anastomoses with an ascending branch of the middle genicular vein. Sometimes it divides into two stems at mid-shaft level, which continue a downward course and finally anastomose with middle genicular derivatives. At the trochanteric fossa the central venous channel is joined by tributaries from the lesser, third, and base of the greater trochanters, as well as a vessel passing down the neck from the head of the femur. It is as though the vein of the trochanteric fossa is a nodal point for vessels draining the bony structures in its vicinity. The division of the middle genicular vein inside the bone into medial and lateral branches, one for each condyle, and also an ascending branch into the inferior metaphysis is usually well visualized, as are also supracondylar veins and veins draining the tip of the greater trochanter into the trochanteric fossa. In the tibiofibula (Pl. 2, fig. 2) a central venous channel runs downwards from the lowest of the subcondylar venous foramina, is joined by the principal diaphyseal nutrient veins, and passes undivided into the inferior epiphysis. Venous radicles from the upper end of the bone pass backwards and join to form other issuing posterior subcondylar veins.

These preparations show how the central venous channel has numerous transverse branches radiating towards the endosteum which, together with the central venous channel, drain the sinusoids of the medulla. Transverse microradiography using 50 % Micropaque injected retrogradely through the veins shows that the medullary sinusoids belong entirely to the venous side of the circulation.

To demonstrate the junction of the arterial with the venous system of bone, thorotrast was injected retrogradely at 60 mm. Hg through the veins, followed by microradiography of transverse sections 1 mm. thick (Pl. 2, figs. 3–6). The medulla shows those venous characteristics already described above, as well as an endosteal line marking the junction of the medullary sinusoids with the cortical capillaries. These traverse the entire thickness of the compactum as profuse straight channels of even calibre. The outer periosteal zone is as abundantly provided with them as is the endosteal. The intermediate zone, on the other hand, often shows an increased vascularity of a plexiform nature (Pl. 2, figs. 3, 6) which, it is suggested, is the site of union of the cortical capillaries with the arterial system described in the previous section.

DISCUSSION

In the course of dissecting the vessels concerned in bone nutrition, it was necessary to delineate the main vascular channels of the rabbit hind-limb. The illustrations of the arterial groups supplying the femur and tibiofibula have been devised to show their origin from the main arteries, which apart from differences in nomenclature deviate from and expand the description given by Krause (1884) in his monograph. The medial circumflex femoral artery, for example, is a large vessel springing directly from the medial side of the femoral artery and not from the A. profunda femoris which, in fact, does not exist in the rabbit. Kistler (1934) described the medial circumflex femoral artery and its acetabular and trochanteric branches, but referred to it as the A. profunda femoris. Huggins & Wiege (1939) quote Kistler's description, and observed the principal nutrient artery to take origin from the lateral circumflex femoral artery. The principal nutrient artery of the tibiofibula, like the corresponding artery of the femur, gives twigs to the periosteum, recalling the periosteal origin in development of the principal nutrient artery of long bones. In man, the arteriae lineae asperae (Barkow, 1868), the longitudinal connexions of the perforating branches of the human A. profunda femoris, as well as the origin of the two principal femoral nutrients from the first and second perforating arteries (Grégoire & Carrière, 1921), are witness to the primarily periosteal nature of this vessel.

In recent years it has been noted that an easy route to the systemic venous system was available for infusions of blood, saline and radiopaque media when these were injected into the cancellous bone of, for example, the medial malleolus. Lamas *et al.* (1946) declared that it was into the 'venous lakes' in the spongiosa that intra-osseous infusions passed, while Harrison & Gossman (1955) showed the ease with which systemic venous filling can be effected by injection of radiopaque material into cancellous bone. Although the principal diaphyseal nutrients are the largest, nevertheless the nutrient arteries at bone extremities are very numerous and are outnumbered by the issuing veins. Their total cross-sectional area must be relatively large, and this accounts for the ease with which intra-cancellous infusions escape into the systemic circulation.

In the visualization of intra-osseous arteries, it soon became apparent that undiluted Micropaque was much to viscous to fill any but the larger vessels. Complete filling as far as the arterioles probably occurs with 50 % suspensions. These demon-

strate that the arterial supply of bone is composed of longitudinal vessels running in the periphery of the medulla and terminating in an arborization of fine vessels at the bone extremities. Transverse branches of the medullary arteries anastomose in the endosteal zone and arborize centrifugally as far as the middle of the cortex but no further. This is in agreement with Clark (1952) who describes the terminal branches of the nutrient artery communicating freely with blood vessels in the Haversian canals, and therefore taking some part in the vascularization of bone tissue. It is unusual for arterial channels to traverse the whole thickness of the compactum. Such vessels, which are few, arise from periosteal arteries, and pass through the cortex to join the endosteal anastomotic network, and thereby augment the medullary arterial system derived from the branchings of the principal nutrient artery and their anastomoses at either end of the bone with the nutrient arteries entering there. In particular, the cortex of the shaft of a long bone does not depend on periosteal arterial twigs for its nutrition. Under conditions which readily demonstrate fine arterial pathways into the cortex from its endosteal aspect, no centripetal vessels can be seen entering the cortex from its periosteal surface, except for the rare vessels mentioned above.

This conclusion is recognized to be at variance with widely held opinion, nor is it suggested here as applying to any other mammal except the rabbit. Nevertheless, certain facts which are usually taken to support a periosteal arterial supply to the cortex of mammalian bone can be used equally well to counter this contention. That living bone oozes blood, rather than bleeding in pulsatile fashion, when stripped of periosteum shows that blood normally *leaves* the surface of the compactum, and is not arterial. Occasional cortical necrosis following periosteal destruction, certainly a rarity in mastoid operations, might well be due to traumatic thrombosis and superimposed infection of superficial cortical capillaries with consequent obstruction to the circulation in the cortex. The role of the periosteum in bone regeneration, and the incorporation of 'blood vessels' from the periosteal vascular network during bone growth in width, as described by Ham (1953), can still be accepted in that the vitality of the osteogenic layer of young periosteum is maintained by the osteogenic capillary layer, fed by the periosteal arteries; the vessels incorporated in the new bone are also capillaries, here called cortical capillaries, in which the blood flows centrifugally into the periosteal network.

Micropaque injections show that the medullary venous system is composed of sinusoids which drain into the central venous channel and its radiating branches. Because the central channel lacks a muscular media (Marneffe, 1951), we may properly speak of a central venous sinus in the medulla.

The thorotrast microradiographs demonstrate that an endosteal venous network is in continuity with fine vessels permeating the cortex, which pass centrifugally to the surface of the compactum where presumably they connect with the osteogenic capillary layer of the periosteum. Because this type of cortical filling has not been obtained in arteriographic or venographic studies using Micropaque, it seems reasonable to conclude that the profuse filling of the cortex in thorotrast microradiographs represents cortical capillaries.

Excluding the nutrient arteries which possess their three tunics, the blood vessels in the cortex have been variously described. Langer (1876) claimed that at least an

artery and a vein can be found in a single canal and often a leash of vessels of capillary size. Testut & Latarjet (1948) insist that they are capillaries, yet nevertheless speak of arterioles entering the cortex from the periosteal network, and also of medullary venous capillaries being in continuity with venules in larger Haversian canals. Lacroix (1951) tersely states that there are one or two capillaries in a Haversian canal, while Marneffe (1951) describes but a single endothelial tube surrounded by a slight adventitia in Volkmann's canals. Maximow & Bloom (1952) state that a Haversian canal carries one or more, usually two, blood vessels which are capillaries or post-capillary venules. Occasionally an arteriole is found in a canal.

It is commonly accepted that 'arteries' enter the cortex from both its periosteal and endosteal surfaces and anastomose with one another in the canal system, the same applying to veins. This implies that there are two vascular lattices in the cortex, which is doubtful; the junction between them is conjectural, and the functional vascular element is left undetermined. In the rabbit, certainly, it seems that there is but one vascular lattice in the cortex composed entirely of simple endothelial tubes, because it is in continuity with the medullary venous sinusoids on the one hand, and the osteogenic capillaries on the other. Into this lattice empty the terminal arborizations of the medullary arteries. Whatever these channels are, arterioles or pre-capillaries, their junction with the functional vascular lattice of the cortex is readily apprehended, and, it is suggested, occurs in the intermediate zone of the cortex. That this lattice should be composed of simple endothelial tubes is to be expected, inasmuch as the functional vascular element of the medulla is of a similar nature, and the cortical canal system is morphologically a medullary space (Leydig, 1856).

It is now possible to attempt a description of the blood flow in bone. Arterial blood from the terminal aborizations in the cortex, derived from the medullary arterial system of bone, empties into a vascular lattice contained in the canals of Havers and Volkmann. Here the circulation is probably very sluggish, and besides movement up and down the diaphysis the blood is capable of shifting into the medulla or periosteum depending on functional variations in opposed muscles and haemopoietic activity in the marrow. Externally the vascular lattice of the cortex connects with the osteogenic capillary layer; internally with the medullary sinusoids. The former route to the systemic veins is direct and probably drains most of the blood circulating in the cortex. The latter route is indirect, through the sinusoids into the central venous sinus, and thence via the nutrient veins at the bone extremities into peri-articular veins.

SUMMARY

1. The gross arterial supply and venous drainage of the rabbit femur and tibiofibula are described and illustrated, and the internal vascularization of bone as revealed by radiography is presented.

2. Nutrient arteries form a medullary system which terminates centrifugally in compact bone as an irregular arborization. The venous system comprises a central venous sinus, its radial branches, and all medullary sinusoids.

3. The arterial and venous systems are united in the cortex by capillaries which permeate it everywhere, and form its functional vascular lattice.

4. Periosteal arteries play no part in the vascularization of compact bone in the adult rabbit. Cortical drainage occurs via the periosteal veins or the medullary sinusoids.

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EXPLANATION OF PLATES

Plate 1

The arterial vascular pattern as shown by radiography or microradiography of the rabbit femur and tibiofibula following injection of various dilutions of Micropaque into the abdominal aorta.

Fig. 1. Femur. 100 % Micropaque injected at 100 mm. Hg. The principal nutrient artery and its main medullary branches. Epiphyseal arteries to the condyles, head and greater trochanter can also be seen.

Fig. 2. Femur. 50 % Micropaque injected at 100 mm. Hg. Complete filling of all arteries within the medulla and cortex of the bone.

Fig. 3. Upper end of femur. 75 % Micropaque injected at 250 mm. Hg. Vascular union can be noted across epiphyseal lines at the head and greater trochanter.

Fig. 4. Tibia. 75 % Micropaque injected at 250 mm. Hg. Transverse branches of main medullary arteries passing to endosteal region of medulla.

Fig. 5. Transverse section of tibia. 50 % Micropaque injected at 100 mm. Hg. Terminal arborization of medullary arteries in inner cortical zone. Microradiogram $\times 20$.

Fig. 6. Transverse section of femur. 50 % Micropaque injected at 100 mm. Hg. Endosteal anastomosis of medullary arteries, and their termination in inner cortical zone. Microradiogram $\times 20$.

Plate 2

Vascular pattern of rabbit femur and tibiofibula as shown by radiography or microradiography following retrograde venous injections (at a pressure of 100 mm. Hg.) of Micropaque or thorotrast.

Fig. 1. Femur. 75 % Micropaque injection. Central venous sinus and its radial medullary tributaries (a cuff of periosteum has been left *in situ* at the centre of the diaphysis).

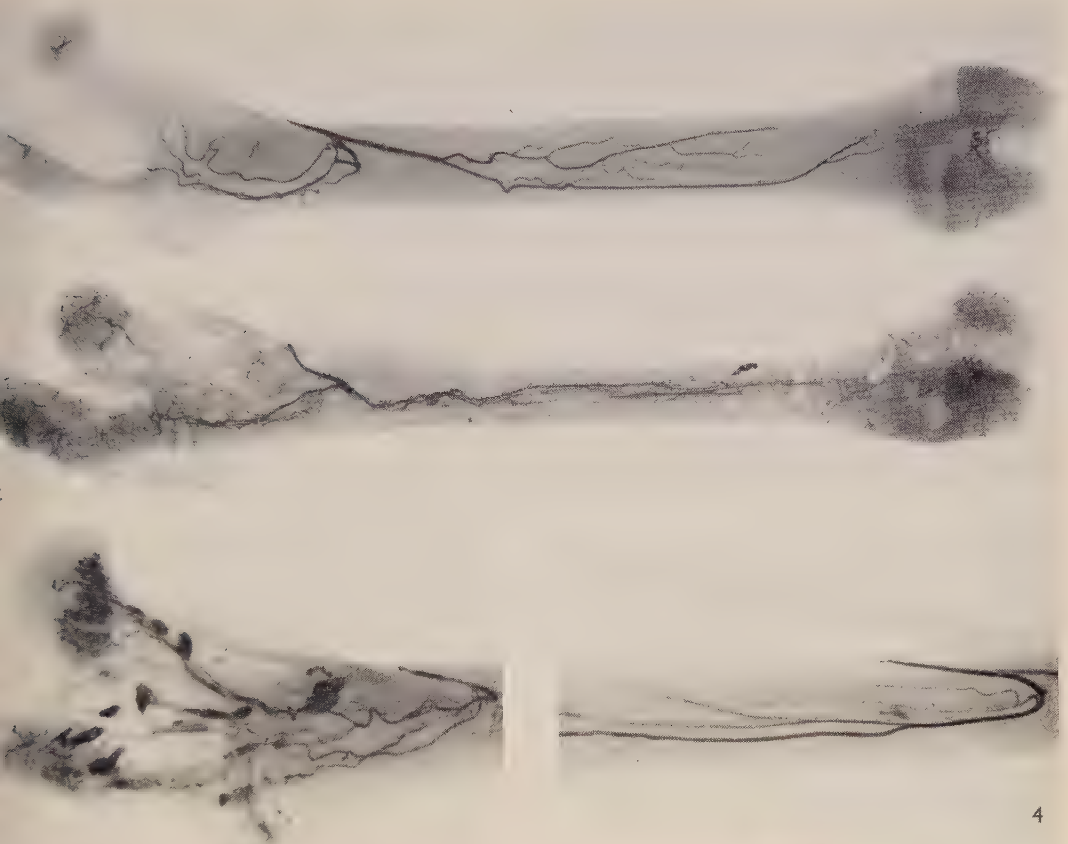
Fig. 2. Tibia. 75 % Micropaque. The central venous sinus and subcondylar veins are clearly shown.

Fig. 3. Tibia. Thorotrast injection. Cortical capillaries and the plexiform arrangement in the intermediate cortical zone may be seen. Microradiogram $\times 22$.

Fig. 4. Tibia. Thorotrast injection. Medullary sinusoids, central venous sinus and its radial branches. Microradiogram $\times 20$.

Fig. 5. Tibia. Thorotrast injection. Central venous sinus, medullary sinusoids, and cortical capillaries. Microradiogram $\times 12$.

Fig. 6. Tibia. Thorotrast injection. Cortical capillaries; endosteal venous line, and medullary sinusoids. Microradiogram $\times 22$.



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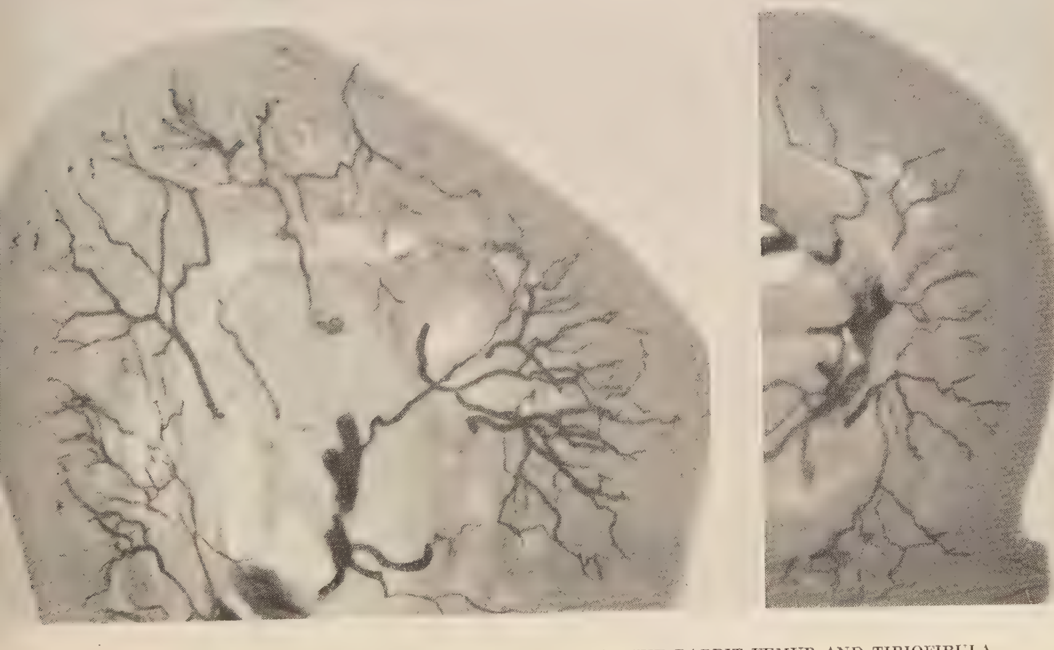

6

BROOKES AND HARRISON—THE VASCULARIZATION OF THE RABBIT FEMUR AND TIBIOFIBULA

(*Facing p.* 72)

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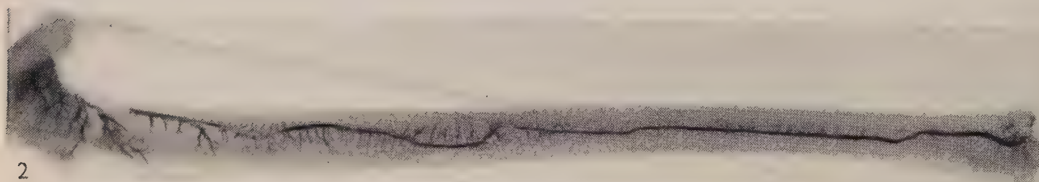

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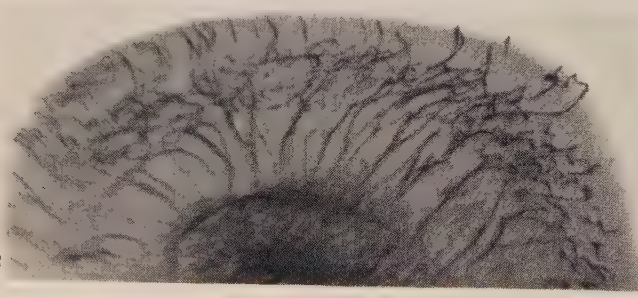

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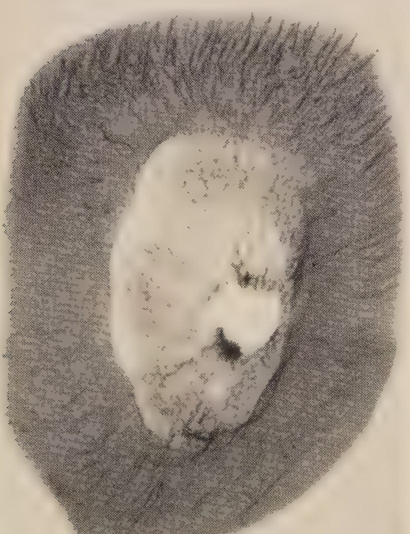

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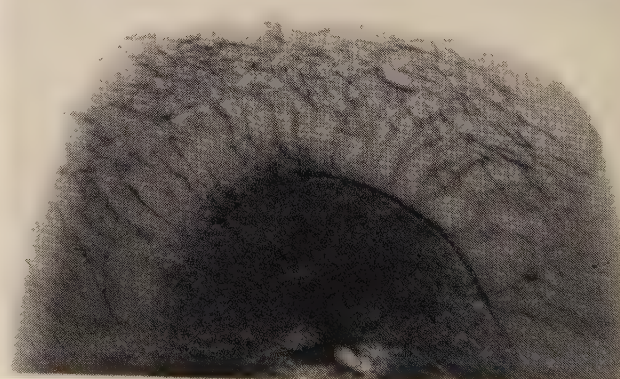

6

BROOKES AND HARRISON—THE VASCULARIZATION OF THE RABBIT FEMUR AND TIBIOFIBULA

FINE STRUCTURAL APPEARANCES IN THE RAT PARATHYROID

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In a study of the parathyroid, the rat gland offers certain advantages in spite of its small size and some difficulty in its routine identification. It is a discrete paired structure situated on the anterolateral aspect of each thyroid lobe, and accessory parathyroid tissue is rarely found in the rat (Hoskins & Chandler, 1925). According to Rosof (1934) and De Robertis (1941) it contains but one histological cell type since the oxyphil cell, a prominent feature of the primate and ungulate glands (Castleman & Mallory, 1935) has not been reported in the rat parathyroid. Within this single cell category both Rosof and De Robertis described 'light' and 'dark' cells, construing these as representing different phases of secretory activity. De Robertis (1941), in a further experimental study on the rat parathyroid, reported an increased number of dark osmiophil cells in states of hypersecretion, while Baker (1945), disputing this contention, found no dark cell increase in parathyroid hyperactivity. Bensley (1947) believed that parathyroid 'dark' cells were dark because of a high lipid content and a low-water content of their cytoplasm in general.

Cowdry (1922) wrote that no intracellular component had been reported which gave any indication of the mode of secretion of the parathyroid cell. Since then Rosof (1934) has described an intracellular osmiophil 'secretory' substance derived, according to him, from Golgi-mitochondrial membrane complexes. In addition to this, basophil bodies (Cowdry & Scott, 1936) and a large juxta-nuclear body (Pappenheimer & Wilens, 1935; Baker, 1942), have also been suggested as being concerned with secretory function.

MATERIAL AND METHODS

Blumenfeld & Rice (1937) found the rat parathyroid to be consistently larger in the female than in the male of the same age, but according to Rosof (1934) there are no cytological sex differences. The present study was made on six female and six male white Wistar rats. Parathyroids were removed by the method of Ingle & Griffith (1949) either under ether or Nembutal anaesthesia: it subsequently appeared that the choice of anaesthetic had no bearing on the quality of the electron micrographs. Having removed a small piece of tissue containing the parathyroid this was as far as possible freed of the surrounding thyroid adnexae. To isolate the parathyroid, the mixed tissue was immersed in a drop of the osmic fixative and since the parathyroid is clearly more osmiophilic than the thyroid, within a few minutes it was conspicuously blackened and so could readily be identified for dissection under a binocular microscope.

Using Dalton's (1955) dichromate-osmic solution (pH 7·2), fixation periods of from 15 to 60 min. were employed. Then followed a brief (5 min.) wash in distilled

water, and rapid ethyl alcohol dehydration (three 5 min. changes of first 95 %, and then 100 % alcohols). After 30 min. in equal parts of monomer and absolute alcohol, the tissue was treated in three 30 min. changes of monomer alone (7 parts of butyl methacrylate: 1 part of methyl methacrylate); and finally in a 0·2 % solution of benzoyl peroxide in the monomer. Some of this last (catalyst-containing) solution was partially polymerized to a syrupy consistency by heat treatment (60° C.) for 30 min. The tissue was then transferred to gelatin capsules containing this pre-polymerized methacrylate and final hardening effected in a 45° C. oven overnight.

Sections in the order of 150–200 Å were cut with glass knives on a Porter-Blum microtome and viewed with a Siemens electron microscope at 80 kV.

RESULTS

It should be said in introduction that low-power electron microscopic observations closely agree with earlier light microscope descriptions of rat parathyroid tissue (Rosof, 1934). The gland is encapsulated, compact and typically epithelial: its parenchymal cells are closely disposed around blood spaces, but clear distinction between arterial and venous capillary channels is not possible in this study. It is advantageous to consider particular cytological features of the gland before attempting a more general assessment, and so the plasma membranes, nuclei, mitochondria, Golgi apparatus, lipid inclusions, and other granular and vesicular components of the cytoplasm, will be treated separately in ensuing sections.

Plasma membranes, subendothelial and intercellular spaces

As already described in several other endocrine tissues (Lever 1956*c*), there is a space contained between the opposed parenchymal cell and capillary endothelium plasma membranes. In addition to a sparse content of collagen fibres this subendothelial space is lined on each side by a semiopaque material which, at the resolution in Pl. 1, fig. 1, appears formless. Similar linings reported by Dempsey & Peterson (1955) in the thyroid were interpreted by them as basement membranes on the grounds that they were argyrophil. It may be that such an identity also applies to the parathyroid (Lever, 1956*c*). As far as can be assessed these 'basement membranes' are unbroken, and although that on the parenchymal side of the subendothelial space may occasionally dip for short distances between the gland cells it is not continued along interparenchymal cell spaces Pl. 1, fig. 2).

On a surface adjacent to a blood channel the parenchymal plasma membrane is uncomplicated and gently curved (Pl. 1, figs. 1, 2); yet where parenchymal cell membranes are opposed to each other these may be extremely convoluted. Thus the intercellular intervals, or spaces contained by these membranes may be correspondingly complex (Pl. 1, figs. 1–3); but long lengths of intercellular space are uncomplicated, the contours of the enclosing membranes being simple. When two surface membranes are closely interdigitating and pursuing a highly irregular course in any plane, then in section portions of both membranes, with the contained intercellular space, may appear to be intracytoplasmic and detached from surface connexion (Pl. 1, fig. 2): such appearances are always found near cell surfaces. Parenchymal plasma membranes lining intercellular spaces are frequently indefinite

and may appear lacking for short distances. At such situations either the cell cytoplasm is in effect freely exposed to an intercellular interval (Fig. 3), or, alternatively, an appearance of this could well be produced if the plane of section be tangential to the plasma membrane. A further feature of the gland cell surface is what may constitute an inward budding of the plasma membrane. The appearances in Fig. 4 strongly suggest a continuity of the plasma membrane with intracytoplasmic membranous components which in section appear as a series of rounded outlines but which might represent a single tortuously curved tube.

The nucleus and nuclear membranes

From a survey of a large number of low-power electron micrographs it appeared that the nuclei in the parathyroid cells are more of less centrally disposed. Their maximal cross-sectional areas were judged to be approximately half those of the whole cells. Irregularities and indentations in nuclear outlines suggest a measure of lobulation.

A peripheral and central zoning of the nucleoplasm (Pl. 2, fig. 6), more marked in some cells than others, showed considerable variation in degree within individual nuclei. This appearance was as well demonstrated in preparations fixed for 15 min. as in those fixed for 60 min. and so should not be construed as a product of faulty fixation. The peripheral zone, variable in thickness and bounded externally by the nuclear membrane, is more electron dense than the central zone. The fine structure of the two zones is basically similar: in a semiopaque background matrix both contain a very fine (140 Åd) vesicular element which cannot be clearly dissociated from a granular element of similar dimensions (Pl. 2, fig. 6). In the peripheral zone there is a much higher concentration of those 'vesiculo-granular' elements.

The nuclear membrane where clearly present is bilaminar (Pl. 2, figs. 9, 10), but over considerable portions of the nuclear surface it may not be clearly represented (Pl. 2, fig. 9). Besides extensive interruptions in the linear outline of the nuclear membrane a smaller type hiatus or 'nucleopore' (Watson, 1955) is also found. At any point where the nuclear membrane is lacking there is free continuity between nucleus and the cytoplasm at large in which there is a tiny vesiculo-granular element of similar dimensions (140 Åd) and appearance to that described in the nucleoplasm. The outer lamina of the nuclear membrane runs a more sinuous course than the inner lamina, and in so doing may sufficiently diverge to outline a bleb or space between the two laminae—such a bleb (Pl. 2, figs. 6, 7) usually has a concentration of vesiculo-granular elements aligned on its cytoplasmic surface. Also it appears highly probable (Pl. 2, fig. 7) that the outer lamina of the nuclear membrane is sometimes continuous with certain cytoplasmic sacs or cisternae (to be described below).

Cytoplasmic granules, sacs and vesicles (other than Golgi components)

All the elements of the granular endoplasmic reticulum, as described by Porter (1953), Palade & Porter (1954) and Palay & Palade (1955), have been observed in this study. Every parathyroid cell contains numerous granules occurring both in discrete fashion in the cytoplasm and also ranged along the outer walls of the membranous components of the endoplasmic reticulum (Pl. 2, figs. 6, 7, 8 and 10).

Some cells or groups of cells are distinctive in containing numbers of much larger granular-walled cisternae or spaces (Pl. 2, fig. 11), while in others only smaller saccular elements and granules are found.

The abundant cytoplasmic granular element observed in the parathyroid is similar in appearance and dimensions to the vesiculo-granular nuclear component already described. In basic structure it is very likely a vesicle the lumen of which is sometimes apparent as an electron-lucid area surrounded by a dense ring of opaque material (Pl. 2, fig. 6, 8), while in some instances the whole body apparently consists of electron-dense material when it then resembles (or is) a granule.

Earlier descriptions (Porter, 1953; Palade & Porter, 1954; Palay & Palade, 1955) of the main features and topography of the endoplasmic reticulum in a number of cell types can in general be applied to the parathyroid. There are however, several points relevant to this tissue which should be made. It is often possible to detect a unilaminar membranous lining to the endoplasmic reticular spaces and this may be incomplete or wanting in some instances (Pl. 2, fig. 8). At these situations the wall of the particular sac or space is composed only of the granular element already described, and at times this covering is much thinner or even wanting when the lumen of the space is in free communication with the cytoplasm at large (Pl. 2, fig. 11). In the endoplasmic reticular spaces is a formless (at the resolution in Pl. 2, fig. 8) semiopaque material resembling the matrix of the general cytoplasm in electron density.

Evidence presented in an earlier section suggests occasional connexions of the plasma membrane on the one hand (Pl. 2, fig. 7) and the outer lamina of the nuclear membrane on the other (Pl. 1, fig. 4) with the walls of intracellular sacs. It is possibly also significant that the elongated membranous elements depicted in Pl. 2, fig. 10, are generally disposed parallel to the nuclear membrane: such bodies might equally be considered as bilaminar sheets with an outer granular covering or as flattened cisternae.

The Golgi apparatus

As far as can be assessed from the present material there is no polarity of the Golgi apparatus in parathyroid cells. Indeed it appears to be randomly and probably widely distributed throughout the cell: as many as three discrete Golgi collections have been seen in section within a single cell.

A Golgi collection usually consists of a lamellar component and what in outline appears to be a vesicular component. These appearances have been observed in a number of tissues (Dalton & Felix, 1954). The lamellar component (Pl. 1, fig. 3) probably consists of bundles of bilaminar sheets which sometimes have terminal expansions thus appearing to communicate with the saccular or vesicular component. This latter component does, however, occur independently of the lamellar membranes and on occasions so much resembles the smallest saccular elements of the granular endoplasmic reticulum as to be indistinguishable from them.

Mitochondria

These are pleomorphic bodies randomly distributed throughout the cytoplasm conforming in many points of their fine structure to the well-known pattern described by Sjöstrand (1953) and Palade (1953). Thus their internal double membranes

are largely arranged as cristae (Pl. 1, fig. 3; Pl. 2, figs. 8, 12 and 13) and their enclosing membranes are seen to be bilaminar in some instances. For the most part, however, mitochondrial limiting membranes were resolved as deeply osmiophile single lines. As in the adrenal cortex (Lever, 1956*a*) mitochondrial limiting membranes in the parathyroid are frequently seen to be deficient (Pl. 1, fig. 3; Pl. 2, fig. 8). At the sites of these deficiencies the internum of the mitochondrion is in communication with the cytoplasm at large.

In parathyroid mitochondria the internal membranes are predominantly but not exlusively disposed as parallel cristae. Other arrangements include irregularly grouped cristae (Fig. 8), filaments and vesicles. As described in the adrenal cortex (Lever, 1955) filamentous membranes in cross-section will appear as a mixture of round or oval outlines while membranous vesicles appear consistently round whatever the plane of section. Some mitochondria have a variegated internal appearance. Thus at one end of the mitochondrion in Pl. 2, fig. 12, these membranes appear as cristae, while at the other their arrangement is probably vesicular.

Mitochondrial vacuolation (Pl. 2, figs. 7, 12) is common but this is more marked in those cells containing numerous large endoplasmic reticular spaces and cisternae (Pl. 2, fig. 11). The vacuolation appears to be a true cavitation within the mitochondrion as there is no circumscribed wall to a vacuole except of course the limiting membrane of the mitochondrion itself.

There is a semiopaque matrix substance contained within mitochondria between their internal membranes and this is indistinguishable from general cytoplasmic matrix wherever the enclosing membrane is deficient. There is considerable variation in the electron density of the internal membranes, which may be lacking or obscured in some (or in parts of some) bodies which are almost certainly mitochondria (Pl. 2, figs. 8, 10 and 12): such bodies are filled with material indistinguishable from that which normally lies between mitochondrial internal membranes and which has been described as matrix substance.

Lipid bodies

In this parathyroid study a number of deeply osmiophile bodies were encountered which, in view of the known lipid content of the gland, may be lipoid in nature. They are present anywhere in the cytoplasm, but it is a strong impression that they are more numerous in that part or parts of the cell juxtaposed to a blood capillary (Pl. 1, fig. 2). As has already been stressed no clear distinction is possible between arterial and venous capillary spaces in this work. In what is possibly a series, these lipid bodies range on the one hand from a form of fairly regular outline with an internal membrane system (Pl. 1, figs. 2, 5) to, on the other hand, an irregular more homogeneous mass (Pl. 1, fig. 2). In the first type of lipid body it cannot be categorically stated that there is or is not an enclosing membrane: certainly in some situations it appears highly probable, while in others there is clearly no definitive covering to these bodies. However, they do possess quite definite *internal* membranes (Pl. 1, fig. 5). These membranes are apparently vesicular or filamentous in disposition and have deeply osmiophile outlines, thus contributing to the mottled appearance of these bodies; which mottling is further enhanced by the presence of

irregular spaces or vacuoles between the internal membranes. The second type of body appears to lie quite freely in the cytoplasm and resembles the lipid aggregates seen in the corpus luteum (Lever, 1956*b*) and the adrenal cortex (Lever, 1955).

DISCUSSION

As in the adrenal cortex in which 'dark' and 'light' cells have been reported both by light and electron microscopy (Hoerr, 1931, Lever, 1955) there is a cell heterogeneity within the rat parathyroid. It has already been stated that in some groups of cells the endoplasmic reticulum is only represented by many granular-walled vesicles and small saccular elements. These cells then appear relatively more electron dense than other groups (within the same tissue) in which are found the largest sacs and cisternae of the endoplasmic reticulum (Pl. 2, fig. 11). Moreover, a pronounced mitochondrial vacuolation is an additional feature in this latter cell type which is less compact and so appears overall less electron dense than the first cell type. Whether or not these differences are sufficient to warrant the use of the terms 'light' and 'dark' cells is a moot point. Certainly one very marked feature of the adreno-cortical light cell, the clusters of thin-walled agranular polyhedral sacs (Lever, 1955), is completely lacking in the normal rat parathyroid. Clearly further investigation is required, but perhaps it is safe to postulate that these two cell types (and the intermediates between them) in the rat parathyroid probably represent differences in physiological state within the gland.

A somewhat surprising feature of the present investigation is the lack of integrity of many of the cell membranes. Thus deficiencies have been reported not only in the mitochondrial envelope but also in the walls of endoplasmic reticular elements, the nuclear membrane, and possibly in the plasma membrane itself. These findings may be helpful in explaining outstanding problems. Without sure knowledge of what constitutes the secretory precursor in the parathyroid it is certain that products of secretion must escape from the cell: if plasma membranes in certain endocrine tissues are normally deficient then secretory products would have easy access to the intercellular spaces and thence to the subendothelial spaces. Deficiencies of plasma membranes have also been observed in the stressed human adrenal cortex (Lever, 1955). The concept of the nucleus actively contributing elements to the cytoplasm is supported by the present findings and although the occurrence of nucleopores has been previously reported by Watson (1955) and others, discontinuities in the linear outline of the nuclear membrane as large as those described here have not been recorded so far as can be ascertained. Deficient mitochondrial envelopes, first demonstrated in the adrenal cortex (Lever, 1956*a*), are also apparently a normal feature of the parathyroid.

Whether or not the lipid bodies are the formative sites of secretory material must remain an open question pending an experimental investigation. Yet they are of interest in themselves. From their appearance they might well be derived from one of three sources: (i) the Golgi vesicular component: this is unlikely since they have not been detected near or in association with obvious Golgi collections. (ii) The cytoplasmic vesiculo-granular component: again this is unlikely in view of the occasional presence of a limiting membrane. (iii) The mitochondria: this is more likely since the filamentous internal membranes of both lipid bodies and mito-

chondria are similar. This is of interest in view of a previous observation that mitochondria are almost certainly the sites of lipid production in the adrenal cortex (Lever, 1955).

SUMMARY

1. An electron microscopic investigation of the rat parathyroid revealed heterogeneous appearances within a single cell type. Some groups of cells contain numbers of granular-walled cisternae and large sacs and many of their mitochondria are vacuolated. These cells are overall less electron dense than others which lack these larger elements of the endoplasmic reticulum and in which only occasional mitochondrial vacuolation is seen. All cells contain small saccular elements of the endoplasmic reticulum and cytoplasmic granules 140 Å in diameter.

2. There is evidence suggesting connexions from both the plasma membrane and the outer lamina of the nuclear membrane with membranous elements of the endoplasmic reticulum.

3. A vesiculo-granular element in the peripheral nuclear zone is freely exposed to the cytoplasm at sites of nuclear membrane deficiency, and resembles the cytoplasmic granular component.

4. Nucleopores and possibly wide deficiencies of the nuclear membrane are common as are deficiencies in the mitochondrial envelope.

5. Lamellar and vesicular Golgi elements are dispersed through the cell as apparently discrete collections.

6. Mitochondrial internal structure is variegated: membranous cristae, vesicles and filaments have been observed.

7. Lipid bodies may contain internal filaments and vesicles suggesting a membranous internum, or can appear as homogeneous drops.

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EXPLANATION OF PLATES

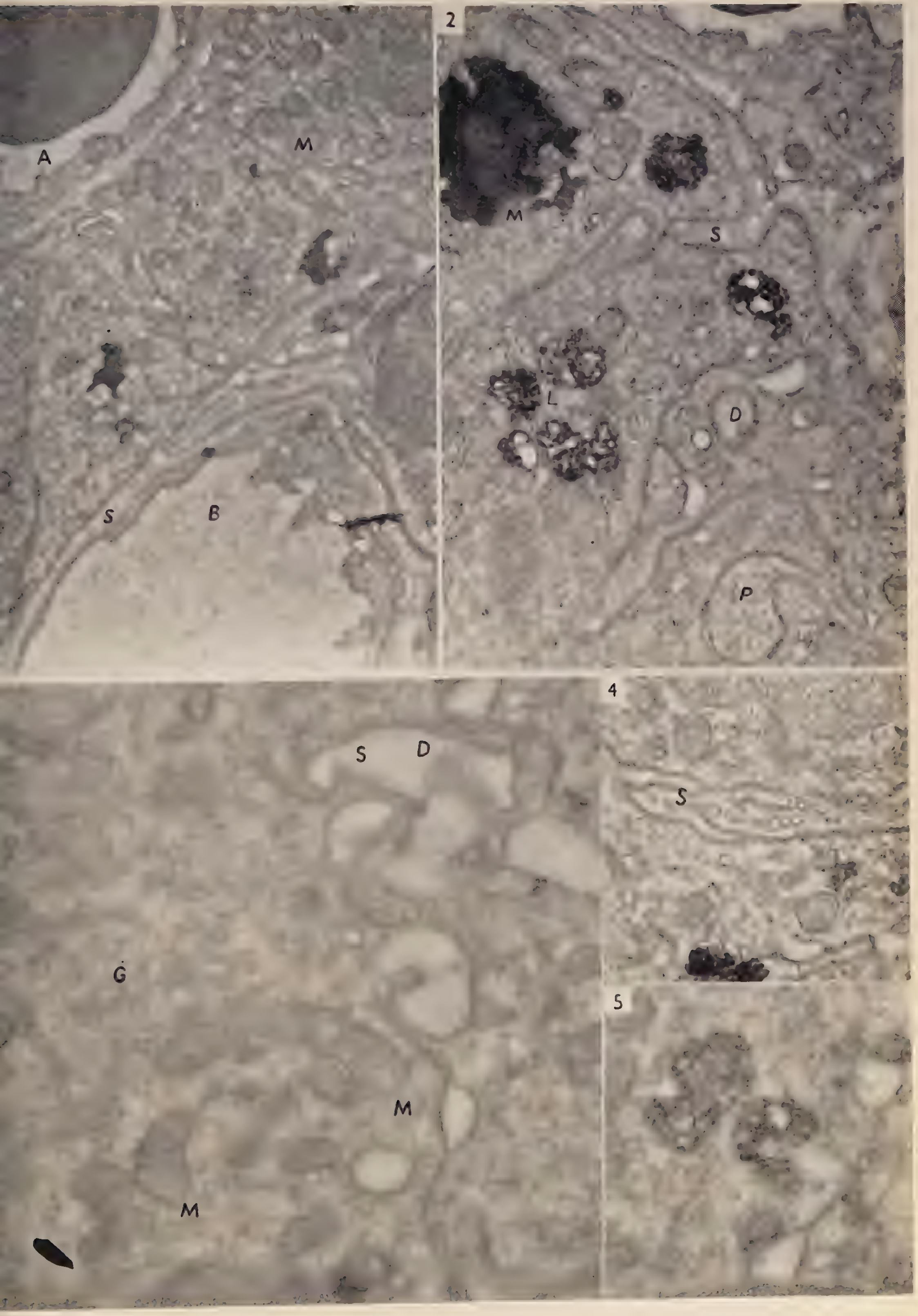
All figures are electron micrographs of the rat parathyroid.

Plate 1

Fig. 1. A group of parenchymal cells between two blood vessels (*A* and *B*). The subendothelial space (*S*) between parenchymal and endothelial plasma membranes is lined on each side by a formless basement membrane. Note tortuous intercellular spaces connecting with subendothelial space. Numerous mitochondrial outlines (*M*) and two lipid aggregates can be seen. ×15,000.

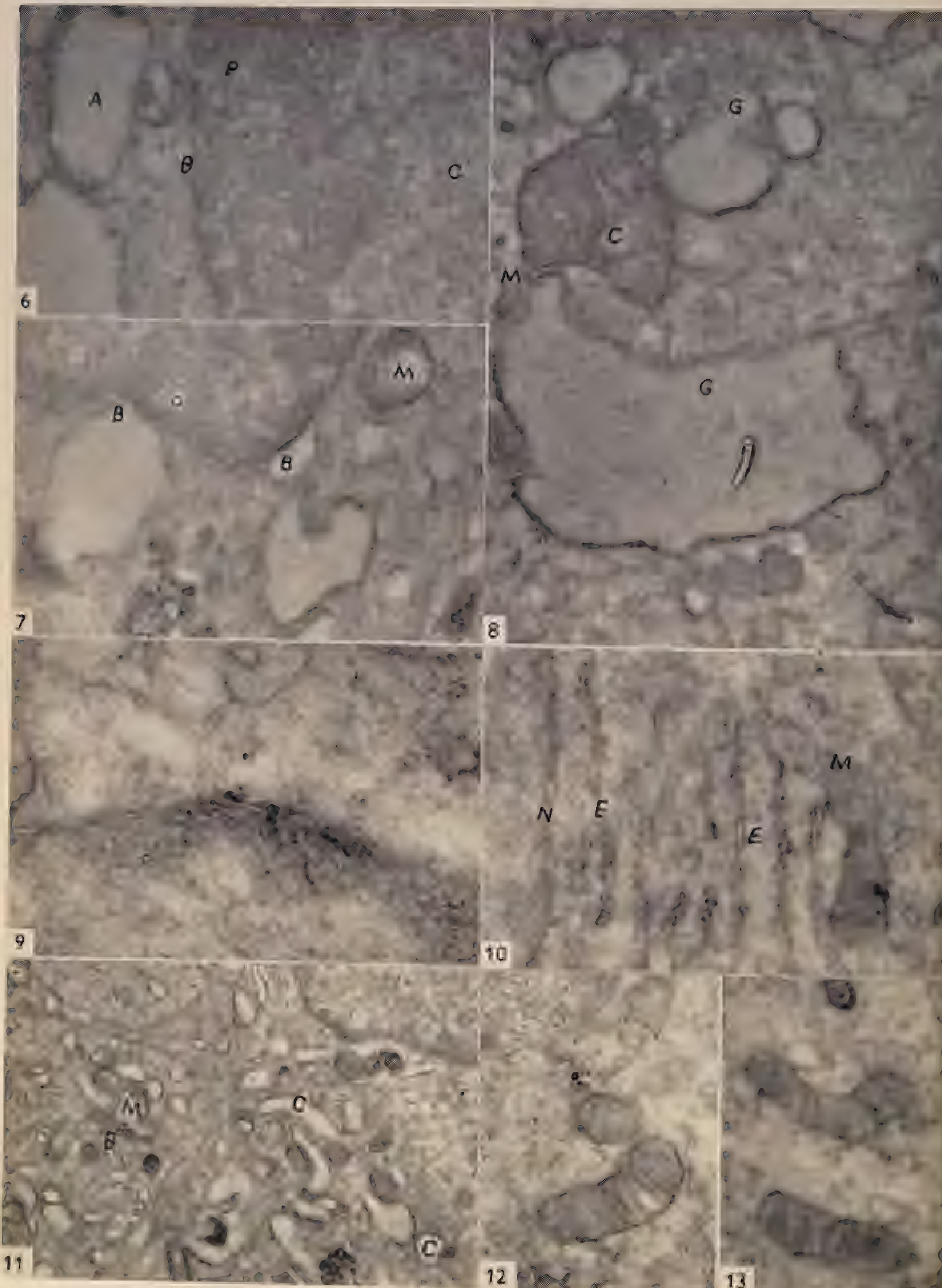
Fig. 2. The basement membrane lining the subendothelial space at *S* dips into but does not continue along the intercellular space. Note extreme interdigitation of plasma membranes at *P*. A different plane of section at *P* could account for an appearance like *D*. Note mottled lipid bodies (*L*) with vesicular internal structures, and the more homogeneous laked lipid mass (*M*). ×25,000.

Fig. 3. The opposed plasma membranes are convoluted and poorly defined at *D*: the intercellular space (*S*) is alternately narrow and wide. Note Golgi collection (*G*) and also deficiencies in the mitochondrial limiting membranes at *M*. Mitochondrial internal membranes are largely grouped as cristae. ×30,000.



LEVER—Fine structural appearances in the rat parathyroid

(*Facing p.* 80)



LEVER—Fine structural appearances in the rat parathyroid

Fig. 4. Appearances at *S* suggest a connexion between the infolded plasma membrane and internal cell membranes. ×25,000.

Fig. 5. These lipid bodies have no obvious limiting membrane but show an internal filamentous or vesicular structure. ×30,000.

PLATE 2

Fig. 6. Peripheral (*P*) and central (*C*) nuclear zones are seen. The outer lamina of the nuclear membrane is blebbed outwards at *B*. Fine vesiculo-granular elements lie in high concentration in the nuclear peripheral zone and along the walls of cytoplasmic cisternae (*A*): they also lie free in the cytoplasm and are aligned along the bleb (*B*). ×30,000.

Fig. 7. Nuclear blebs (B) as in fig. 6 are here probably communicating with cytoplasmic cisternae. Note vacuolated mitochondria (*M*). ×30,000.

Fig. 8. Several cisternae lack a definitive membranous wall and at *G* are lined only by a vesiculo-granular element. In the large mitochondrion note irregular cristae (*C*) and the deficient limiting membrane at *M*. ×30,000.

Fig. 9. To the left the nucleus (bottom) is confined by a bilaminar membrane. To the right nucleoplasm and cytoplasm are freely apposed. ×50,000.

Fig. 10. *N* is the nuclear membrane and *E* are long bilaminar endoplasmic reticular elements parallel to it. Note the body *M* having an appearance suggestive of a mitochondrion with a faint vesicular or filamentous internum. ×40,000.

Fig. 11. This low-power micrograph shows cells with an overall low electron density containing numbers of cisternae and large sacs (*C*), some of which appear to lack a definite wall. Many of the mitochondria (*M*) are vacuolated. Note mitochondrion (*B*) with vesicular internal membranes. × 15,000.

Fig. 12. In the upper part of this mitochondrion internal membranous cristae are apparent while below there are a few faint vesicular outlines within the matrix material. ×45,000.

Fig. 13. To show cristae mitochondrales. ×40,000.

THE DORSAL TRIGEMINAL TRACT IN THE RHESUS MONKEY*

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Although considerable attention and research have been directed to the study of the secondary trigeminothalamic fibre systems because of their great theoretical and practical importance, accurate knowledge of these systems is still incomplete and controversial. The present communication concerning the dorsal trigeminal tract in the rhesus monkey is a report of significant incidental observations made during a study of the brachium conjunctivum.

The following experimental data support the existence of an uncrossed dorsal trigeminal fasciculus originating from the chief sensory nucleus of the trigeminal nerve and probably excludes the possibility of the existence of an uncrossed ascending component of the brachium conjunctivum, as described by Carrea & Mettler (1954).

MATERIAL AND METHODS

Sixteen immature rhesus monkeys weighing from 2800 to 3800 g. were used. In seven of these animals attempts were made to produce localized lesions in various parts of the brachium conjunctivum by stereotaxic methods (Carpenter & Whittier 1952). Electrodes were introduced into the brain in the frontal region and passed ventrocaudally into the dorsolateral portion of the mesencephalon. Successful lesions limited to the brachium conjunctivum were produced in five animals (2118, 2124, 2137, 2190 and 2191). In one (2124) of these animals the lesions destroyed portions of the brachium conjunctivum ventrally and extended caudally into the dorsal part of the chief sensory nucleus of the trigeminal nerve.

In eight monkeys the brachium conjunctivum was sectioned with a small knife caudal to the inferior colliculus. None of these lesions was restricted to the brachium conjunctivum. Adjacent structures commonly involved were the inferior colliculus, the trochlear nerve, the ventral spinocerebellar tract, the lateral lemniscus and dorsal trigeminal tract. In one additional animal (2413) an attempt was made to remove the entire cerebellum by suction. Although small areas of cortex remained about the middle cerebellar peduncle, the removal was virtually complete.

Observations regarding postoperative animal behaviour and neurologic status were recorded at various intervals. Cinematographic records were taken of noteworthy physiologic phenomena. At the conclusion of the observation period (always at least 14 days) animals were examined in a restraining chair, anaesthetized with intravenous Nembutal and perfused with 500 ml. of 10% neutral formalin. The

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brains and portions of the spinal cords were removed and placed in 10% neutral formalin for further fixation. At a later time the brains were cut into slabs approximately 2 mm. thick, perpendicular to the axis of the brain stem. Sections of the brains and portions of the spinal cord were prepared according to the Marchi method. Sections were cut serially at 23μ and every 10th section was mounted. Sections of the cerebellum were embedded in celloidin (sometimes paraffin), sectioned serially and stained with cresyl violet.

RESULTS

Lesions involving the chief sensory nuclei of the trigeminal nerve. In one animal (2124) bilateral stereotaxic lesions destroyed portions of the chief sensory nucleus, as well as ventral portions of the brachium conjunctivum.

Following surgery the animal was alert but hypokinetic and seemed to have persistent hiccups. On the second postoperative day, it was noted that the animal broke its food into small pieces with its hands and fed directly from the floor. It was obvious that the animal had difficulty chewing food. The jaw hung partially open and could not be closed. Dislocation of the jaw during surgery was suspected, but examination revealed only weakness of the muscles of mastication bilaterally. From the 2nd to the 11th postoperative day the animal tore food into small bits with his teeth, because he was unable to chew. After this time, weakness in the muscles of mastication was no longer apparent. At examination on the 21st postoperative day, prior to sacrifice, only slight pupillary inequality was found. Cranial nerves appeared functionally intact and no sensory deficit could be detected by pin prick or cotton-wool in the trigeminal distribution or elsewhere. Resistance to passive movement seemed diminished in the muscles of the lower extremities. The animal was sacrificed by perfusion with 500 ml. of 10% neutral formalin.

Descriptions of the lesion. On the right side the rostral limit of the lesion began at the level of the trochlear nerve decussation and was located in the ventrolateral third of the brachium conjunctivum which it completely destroyed. At slightly more caudal levels the lesion expanded to destroy some fibres of the lateral lemniscus and the ventral spinocerebellar tract. The dorsal two-thirds of the brachium conjunctivum was preserved and entirely free of Marchi degeneration. Caudally the lesion entered the dorsomedial part of the chief sensory nucleus of the trigeminal nerve where this structure lies ventral to the brachium conjunctivum. Fibres of the mesencephalic root of the trigeminal nerve were interrupted as well as secondary fibres issuing from the chief sensory nucleus. Intense Marchi degeneration was scattered throughout the masticator nucleus and was present in parts of the superior vestibular nucleus.

The lesion on the left side began at the level of the inferior colliculus slightly dorsal to the middle third of the brachium conjunctivum. It expanded caudally to destroy the most ventral and mesial part of the middle thirds of the brachium conjunctivum as well as all structures between the medial concavity of the superior cerebellar peduncle and the lateral border of the periaqueductal gray. The caudal extent of the lesion was situated dorsomedial to the chief sensory nucleus. Fibres of the mesencephalic root of the fifth nerve were interrupted and part of the superior vestibular nucleus was destroyed.

Marchi degeneration. Moderately heavy Marchi degeneration was found in the principal trigeminal root fibres bilaterally; this degeneration was traced peripherally through the lateral portion of the pons on both sides. In the masticator nuclei dense black Marchi granules were seen bilaterally. Clear distinct Marchi degeneration emanating from the chief sensory nuclei and from the area dorsomedial to the chief sensory nuclei was seen. These degenerated fibres formed a triangular-shaped bundle along the medial concavity of the brachium conjunctivum and constituted the first part of the dorsal trigeminal tract. A few degenerated fibres from the area of the lesion passed medially ventral to the genu of the seventh nerve and appeared to enter the medial longitudinal fasciculus; some of these fibres crossed the median raphe to the opposite side. The latter fibres were considered to be vestibulomesencephalic fibres from the superior vestibular nucleus, but a few may have originated in the chief sensory nucleus. No fibres from the chief sensory nucleus were seen to enter the medial lemniscus on either side.

At levels through the inferior colliculus the fibres of the brachium conjunctivum began to move ventromedially preparatory to decussating. The fibres of the brachium conjunctivum passed through the dorsal trigeminal tract from dorsal to ventral and from lateral to medial. Thus, below these levels the dorsal trigeminal tract was medial to the brachium conjunctivum and above them it was dorsal. In this area it was difficult to follow the degeneration in the dorsal trigeminal tract because of co-existing degeneration in the brachium conjunctivum. At the level of the trochlear nuclei the dorsal trigeminal tract was again isolated and distinct, dorsolateral to the medial longitudinal fasciculus. This tract became more clearly separated from the medial longitudinal fasciculus in the region of the third nerve nuclear complex. The tract maintained this position until levels through the posterior commissure were reached. As the tract ascended it moved slightly lateral away from the periaqueductal gray. Above the level of the posterior commissure the tract was a less compact bundle of fibres compared with lower levels. Fibres of the fasciculus retroflexus traversed the dorsal trigeminal tract. Some of the fibres of the dorsal trigeminal tract were medial to the fasciculus retroflexus and some were lateral to it. At this level the dorsal trigeminal tract made an abrupt lateral bend and passed ventrolaterally through the medial part of the centromedian nucleus to enter the arcuate nucleus of the thalamus. It was impossible in this preparation to determine whether some of the fibres terminated in the centromedian nucleus or merely traversed this structure *en route* to the arcuate nucleus. Degeneration was most dense in the arcuate nuclei.

The fibres of the mesencephalic root of the trigeminal nerve were interrupted completely on the left side and in part on the right by the lesions. Degenerated fibres of the mesencephalic root were seen to pass rostrally into the lateral border of the periaqueductal gray. At the level of the trochlear nerve decussation the fibres shifted dorsally and came into apposition to the fibres of the trochlear nerve dorsally and the brachium conjunctivum laterally. Some of the fibres appeared to enter the brachium conjunctivum.

Very slight Marchi degeneration was seen in the descending root of the trigeminal nerve bilaterally. Such degeneration as was present consisted mainly of fine granules with occasional scattered coarse granules. This type of degeneration could be

followed only to the level of the inferior olivary nuclear complex and was not impressive. No detectable degeneration was seen emanating from the spinal nucleus of the trigeminal nerve.

Marchi degeneration of the brachium conjunctivum resulting from these lesions could be followed rostrally through the decussation, into the red nuclei, the prerubral fields, and into fields H_1 and H_2 of Forel. From these sites degeneration could be followed into the lateroventral nucleus of the thalamus and into the globus pallidus via both the dorsal and ventral divisions of the ansa lenticularis.

Lesions of the dorsal trigeminal tract. Unequivocal Marchi degeneration was found unilaterally in the dorsal trigeminal tract in five animals (2116, 2122, 2123, 2129 and 2179) following surgical lesions of the brachium conjunctivum. In rhesus 2130 bilateral degeneration was found in this tract after bilateral lesions of the brachium conjunctivum. In two additional animals (2119 and 2210) with unilateral surgical section of the brachium conjunctivum 232 and 222 days prior to sacrifice, the dorsal trigeminal tract was fully degenerated and could not be seen in Marchi-stained preparations on the side of the lesions.

Fibres of the dorsal trigeminal tract were interrupted medial to the brachium conjunctivum at levels slightly below the trochlear nerve decussation in these animals. In rhesus 2123 the lesion extended caudally into the dorsal part of the chief sensory nucleus. Degeneration of the dorsal trigeminal tract was clearly seen at the level of the trochlear nuclei and was on the same side as the lesion in every instance. No degeneration was seen in the contralateral dorsal trigeminal tract or in the medial lemniscus in any of these animals. Marchi degeneration in these cases was more impressive and heavier than that seen in rhesus 2124, presumably because the entire tract was destroyed. The ascending course of the degenerated fibres was the same as previously described. Since this was the only thalamic degeneration on the side in question in five of these monkeys (degenerated fibres of the brachium conjunctivum having crossed to the opposite side in the brachial decussation), it was easy to follow the course of the fibres.

In none of these animals was it possible to detect impairment of tactile sense over any part of the face. No special testing procedures were employed, but it is possible that with more careful testing some sensory deficit might have been found.

Total removal of the cerebellum. In rhesus 2413 with virtual total removal of the cerebellum no degeneration was seen in the dorsal trigeminal tracts. The brachium conjunctivum was completely degenerated bilaterally.

Stereotaxic lesions of the brachium conjunctivum. In those animals (2118, 2137, 2190 and 2191) with lesions restricted to various parts of the brachium conjunctivum, no degeneration was found in the dorsal trigeminal tracts.

DISCUSSION

The controversy concerning the existence and nature of the dorsal trigeminal tract has continued over a number of years. This tract, first described by Forel (1877), has been referred to as Forel's Haubenfaszikel (tractus fasciculorum Foreli, not to be confused with Forel's Haubenfeldern H, H_1 and H_2) but many authors prefer to designate it as Wallenberg's fasciculus since he first drew attention to it as a

trigeminothalamic pathway. The tract has been considered to be crossed by Hösel (1892), Wallenberg (1896, 1905), Spitzer (1899), van Gehuchten (1901) and Winkler (1921); Hatschek (1902), Lewandowsky (1904), Kohnstamm (1910), von Economo (1911), Papex & Rundles (1937), Hassler (1948), Verhaart (1949), Papez (1951) and Russell (1954) have considered it to be uncrossed. Both crossed and uncrossed fibres of the dorsal trigeminal tract were described by Cajal (1904), Woodburne (1936) and Walker (1939). Most authors have concluded that this tract represents a trigeminothalamic pathway, but this opinion is not unanimous. Fibres of this tract were reported to arise from the spinal nucleus of the fifth nerve by Hösel (1892), Wallenberg (1896), Spitzer (1899) and van Gehuchten (1901). The chief sensory nucleus of the trigeminal nerve was stated to be the origin of these fibres according to Hatschek (1902), Kohnstamm (1910), von Economo (1911), Winkler (1921), Papez & Rundles (1937), Walker (1939) and Papez (1951). Cajal (1904) concluded that the fibres arise from both the spinal and the chief sensory nuclei of the trigeminal nerve. Hassler (1948), on the basis of myelogenetic studies and the examination of varied types of human pathological material, concluded that Forel's fasciculus was a direct secondary vestibulothalamic pathway, but in the investigations of Gray (1926), Rasmussen (1932), Buchanan (1937) and Ferraro, Pacella & Barrera (1904) no degeneration in this tract was observed following lesions in various vestibular nuclei. Verhaart (1949) identified fibres of this tract as contributing to the dorsal and lateral part of the central tegmental fasciculus in man and monkey. Lewandowsky (1904) felt this fibre tract originated in the reticular formation of the medulla and pons. Studies done on cats by Russell (1954) indicated that no dorsal trigeminal tract, either crossed or uncrossed, could be demonstrated following lesions limited to the sensory nuclei of the trigeminal nerve. He expressed the opinion that the fibres usually described as the dorsal trigeminal tract actually represent an uncrossed ascending lateral reticulothalamic pathway. Bürgi (1955) concurred in this view, stating that these fibres probably were part of the ascending reticular activating system. Von Economo (1911) believed that taste was mediated by the dorsal trigeminal tract.

Berry, Anderson & Brooks (1956), in electrophysiologic studies in cats, located a dorsal trigeminal tract lateral to the medial longitudinal fasciculus in the mid-brain which conducted both ipsilateral and contralateral impulses following stimulation of the infraorbital nerve by single shocks. The earlier investigations of Mountcastle & Henneman (1952) indicated that ipsilateral tactile impulses from the face and mouth projecting to the medial part of the arcuate nucleus in the monkey could be demonstrated by electrophysiological methods. These data would seem to support the existence of an uncrossed dorsal trigeminal tract.

The material presented here demonstrates that trigeminothalamic fibres arise from at least the dorsomedial part of the chief sensory nucleus and ascend uncrossed in the dorsal trigeminal tract. The ascending fibres pass medial to the brachium conjunctivum where they appear as a triangular-shaped bundle. At the level of the inferior colliculus the fibres of the brachium conjunctivum pass through those of the dorsal trigeminal tract from dorsal to ventral and from lateral to medial. At the level of the trochlear nucleus and the decussation of the brachium conjunctivum the fibres of the dorsal trigeminal tract lie lateral to the medial longitudinal fasciculus

at the border of the periaqueductal gray. Fibres ascend in this position to the level of the fasciculus retroflexus where they make an abrupt ventrolateral bend and sweep into the medial part of the arcuate nucleus of the thalamus. No crossed degeneration was seen in the opposite dorsal trigeminal tract or in the medial lemniscus.

Carrea & Mettler (1954), in their study of the brachium conjunctivum in monkeys, observed uncrossed degenerated fibres ascending to the medial part of the arcuate nucleus of the thalamus in four animals (1120, 1122*a*, 1128 and 1135). Lesions in these animals were inflicted in the same manner as in in the currently reported cases. These authors, however, considered the degeneration in the dorsal trigeminal tract to represent an uncrossed ascending component of the brachium conjunctivum which heretofore had not been described. This 'component of the brachium conjunctivum' was described as originating from the dorsomedial portion of the superior cerebellar peduncle at the level of the inferior colliculus. The tract was stated to form in the following manner: 'When these fibres reach the outer crescent of the medial longitudinal fasciculus, where the uncrossed vestibulomesencephalic and the tectobulbar and the tectospinal tracts are located, this bundle becomes detached from the body of the brachium and remains lateroventral to the medial longitudinal fasciculus' (pages 584–6). The course of the tract was thereafter identical with that described in the current cases for the dorsal trigeminal tract. Since the only portions of the brachium conjunctivum destroyed by the lesions in rhesus 2124 (in addition to parts of the chief sensory nuclei of the trigeminal nerve) were located ventrolaterally, it seems unlikely that these fibres could become 'detached from the body of the brachium' as described above. If the fasciculus in question contained fibres of cerebellar origin passing in the brachium conjunctivum, one would expect that a few degenerated fibres might be seen in those cases with stereotaxic lesions of the brachium conjunctivum and that clear distinct degeneration would certainly be present if the entire cerebellum were removed. No Marchi degeneration in the dorsal trigeminal tract was seen in any of these cases.

Further evidence of another type suggests that the fibres of the dorsal trigeminal tract are in no way related to the brachium conjunctivum. In a series of fifteen rhesus monkeys (Carpenter & Taber, 1956) with lesions of the deep cerebellar nuclei, no degeneration in the dorsal trigeminal tract (uncrossed ascending limb of the brachium conjunctivum of Carrea & Mettler) was found, although degeneration was seen in all parts of the brachium conjunctivum.

SUMMARY AND CONCLUSIONS

This study of the dorsal trigeminal tract in the rhesus monkey was based upon significant incidental observations made during an investigation of the brachium conjunctivum. Sixteen animals were used for this study. Complete lesions of the brachium conjunctivum were produced by surgical section in eight animals and localized partial lesions were produced stereotaxically in five of seven monkeys. In one of the latter animals lesions extended into the chief sensory nucleus of the trigeminal nerve. In another animal the entire cerebellum was removed. Degenerations resulting from these lesions were studied in Marchi serial sections.

The following conclusions were drawn:

1. Secondary trigeminal fibres arising from the dorsal part of the chief sensory nucleus of the trigeminal pass dorsomedially in the upper pontine tegmentum to form the dorsal trigeminal tract which initially lies medial to the brachium conjunctivum. Fibres of the brachium conjunctivum pass through the dorsal trigeminal tract at the level of the inferior colliculus preparatory to decussating.

2. The fibres of the dorsal trigeminal tract ascend lateral to the periaqueductal gray to the level of the fasciculus retroflexus, where they make an abrupt ventrolateral bend and sweep through the medial part of the centromedian nucleus and into the medial part of the arcuate nucleus of the thalamus.

3. The dorsal trigeminal tract is an uncrossed ascending pathway from the chief sensory nucleus of the trigeminal nucleus.

4. No secondary trigeminal fibres originating from the dorsomedial part of the chief sensory nucleus appear to ascend in association with either the medial lemniscus or the spinothalamic tract.

5. The fibres of the dorsal trigeminal tract do not appear to constitute an 'uncrossed ascending limb of the brachium conjunctivum' as has been suggested by certain authors.

6. It is possible that the dorsal trigeminal tract may be a compound fasciculus containing ascending fibres originating from sources other than the chief sensory nucleus of the trigeminal nerve, but the proportion of such fibres, if they exist, is small.

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EXPLANATIONS OF PLATES

PLATE 1

Fig. 1. Rhesus 2124. Section through the pons at the level of the chief sensory nuclei of the trigeminal nerve showing stereotaxic lesions. The lesion on the right destroyed the dorsomedial portion of the chief sensory nucleus and the ventrolateral third of the brachium conjunctivum. The lesion on the left destroyed the ventromedial fibres of the brachium conjunctivum, fibres of the mesencephalic root of the trigeminal, fibres of the dorsal trigeminal tract and a small part of the chief sensory nucleus. Marchi, ×6.

Fig. 2. Rhesus 2124. Photomicrograph demonstrating bilateral degeneration in the dorsal trigeminal tract resulting from lesions shown in Fig. 1. Marchi, ×12.

PLATE 2

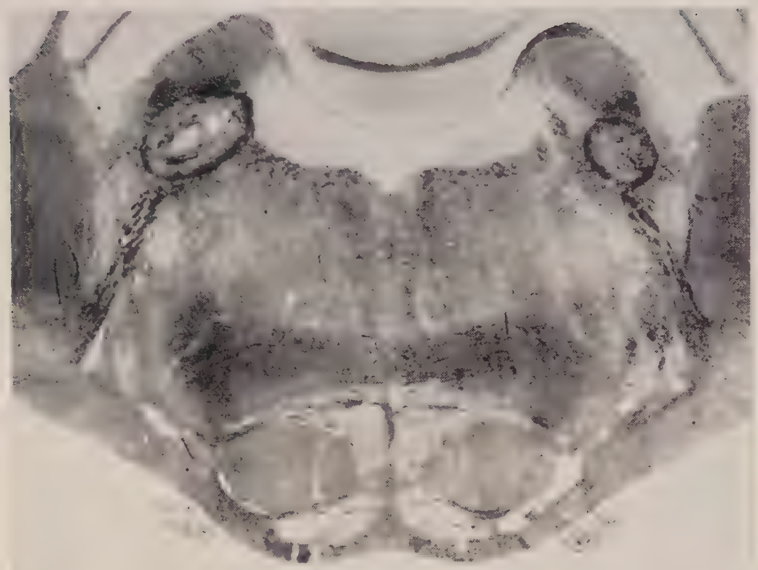
Fig. 3. Rhesus 2123. The brachium conjunctivum and the dorsal trigeminal tract were sectioned on the left side. Degeneration is present in the uncrossed dorsal trigeminal tract (left) and in the brachium conjunctivum above the decussation (right). Marchi, ×7.

Fig. 4. Rhesus 2123. The degenerated dorsal trigeminal tract on the left has become a less compact bundle at the level of the posterior commissure. Degenerated fibres of the brachium conjunctivum can be seen traversing the rostral portion of the red nucleus on the right. Marchi, ×10.

Fig. 5. Rhesus 2123. Degenerated fibres of the left dorsal trigeminal tract can be seen sweeping ventrolaterally through the centromedian nucleus into the medial part of the arcuate nucleus of the thalamus. Marchi, ×14.

Plate 3

Fig. 6. Semi-schematic drawings showing the course of the dorsal trigeminal tract from its origin in the chief sensory nucleus of the trigeminal nerve to its termination in the medial part of the arcuate nucleus of the thalamus. The uncrossed fibres of the dorsal trigeminal tract are represented by coarse black dots at the following levels: A, chief sensory nucleus of the trigeminal nerve; B, Trochlear nerve decussation; C, inferior colliculus (note that fibres of the brachium conjunctivum pass through the dorsal trigeminal tract); D, trochlear nuclei; E, third nerve nuclear complex; F, Fasciculus retroflexus (note fibres of the dorsal trigeminal tract sweeping into the medial part of the arcuate nucleus).



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CARPENTER—Dorsal trigeminal tract in the rhesus monkey

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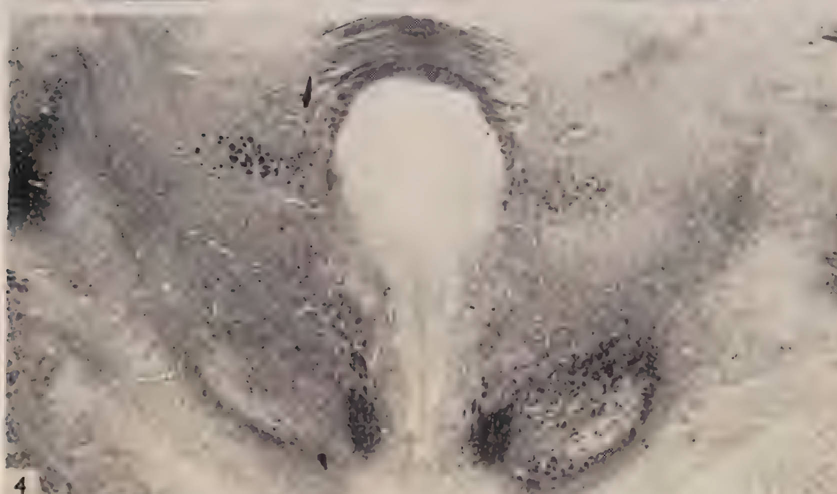

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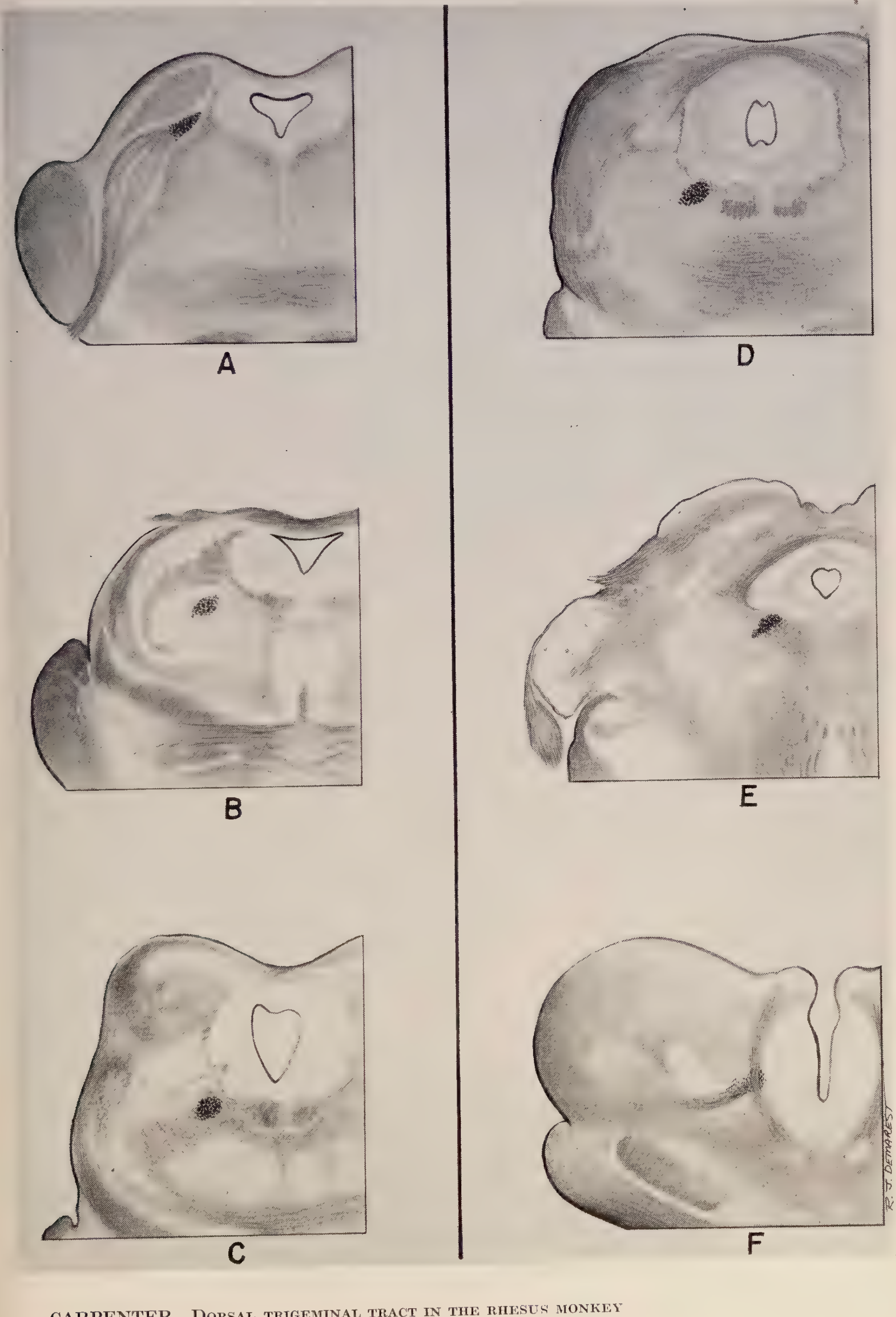
CARPENTER—DORSAL TRIGEMINAL TRACT IN THE RHESUS MONKEY



CARPENTER—Dorsal trigeminal tract in the rhesus monkey

DEGENERATION IN THE HYPOTHALAMIC CONNEXIONS OF THE ALBINO RAT

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INTRODUCTION

The poster or hypothalamus has been studied by a variety of methods. Stimulation of this region gives rise to sympathetic effects (Ranson & Magoun, 1939; Hess, 1954), while bilateral lesions can produce somnolence (Ranson, 1939; Nauta, 1946) or interfere with temperature regulation (Ranson & Magoun, 1939). Moruzzi & Magoun (1949) have included the posterior and dorsal part of the hypothalamus in the brain-stem activating system, and Porter (1954) has shown that the posterior hypothalamus plays an important part in the anterior pituitary response to stress stimuli. Anatomical studies show that there are three important fibre systems in this part of the hypothalamus: the mamillary system of connexions, the periventricular system and the medial forebrain bundle. The mamillary system of connexions has been studied by a large number of observers (see Guillery, 1956) and the descending fibres of the medial forebrain bundle and periventricular system have been described by Beattie, Brow & Long (1930) and Magoun (1940), among others. Ascending fibres that pass through this region have been described by Nauta (1946) and Morin (1950), but the course and the termination of these fibres has received little attention.

In the present investigation these three fibre systems and their mutual interrelations have been studied. Lesions were placed in the posterior hypothalamus and the subsequent degeneration studied by the method of Nauta & Gygax (1954). The efferent mamillary tracts are clearly distinct from the other hypothalamic connexions and have been described in the first part of this paper. The degeneration in the medial forebrain bundle and the periventricular system has been described in the second part. The two parts have been included in a single paper, not only because the degeneration was produced by a single set of lesions but also because the pattern of this degeneration has shown a number of interrelationships between the mamillary and the premamillary hypothalamic connexions.

The fibres that pass from the premamillary hypothalamus to the thalamus have not been described in this paper since the material available is not sufficient to give a clear picture of this projection system.

MATERIALS AND METHODS

Nine rats with lesions in the hypothalamus and four rats with lesions in the midbrain have been used. The hypothalamic lesions were made through a ventral, parapharyngeal approach (see Ingle & Griffith, 1942), with a small ophthalmic knife that was passed through the hypophyseal fossa after most of the gland had been

removed. Two of the midbrain lesions were made through a similar ventral approach with the burr hole placed further caudally. In this position particular care has to be taken to avoid the basilar artery. The other two midbrain lesions were made by inserting a curved ophthalmic knife under the cerebellum and through the floor of the fourth ventricle into the dorsal parts of the midbrain and pons. Ether anaesthesia was used for all the operations.

The animals were allowed to survive for 3–7 days and were then killed under ether anaesthesia by perfusion with 10 % formol saline. The brains were embedded in Carbowax and were cut serially at 15–18 μ. Every 5th section was stained with cresyl violet and most of the other sections were stained by the method of Nauta & Gygax (1954). The appearance of the Nauta sections depends upon the precise times and solutions that have been used. Using carbowax-embedded material it is difficult to suppress the staining of normal fibres completely, and normal fibres were apparent in a number of the sections (see, for example, Pl. 2, fig. 11). Such normal fibres can, however, always be clearly distinguished from the degenerating fibres. Only fibres which had broken up clearly and had formed distinct beads and granules were regarded as degenerating. As a further check on the degeneration appearances control sections from unoperated animals were stained together with the experimental sections and only those appearances that could be traced serially through the block and could not be matched on control material have been accepted as degeneration.

During the present investigation it has been found that different lesions can produce patterns of degeneration having a similar distribution but showing marked differences in terms of density of degeneration. The apparent density of the degeneration depends upon the thickness of the sections, upon the survival time of the animals and upon the precise times and solutions that were used during the staining procedure. Although the variation due to the last factor can be reduced by staining a large number of sections from any one block, which gives a majority of sections showing reasonable consistency, it is not possible to give an accurate estimate of the density of degeneration in any region. In the following descriptions the terms 'heavy', 'dense', 'moderate' and 'sparse' have been used to indicate, in order, decreasing densities of degeneration. They have been used as comparative terms within a single region and have no absolute value. The difference between the degeneration described by any two terms has been found with reasonable consistency and, in a number of instances, has shown an important relationship to the position of the lesion.

Table 1 gives the period of survival, the general position of the lesion and the plane of the sections for each of the thirteen animals. The majority of the brains were cut parasagittally, but in Text-figs. 1, 2 and 4, the position of the lesions and the septal degeneration have been shown schematically on frontal sections. The reconstructions from the parasagittal to the frontal plane have been made in terms of nuclear groups and fibre tracts, not in terms of absolute measurements.

Table 1

Rat no.	Survival period (days)	Plane of sections	Approximate position of lesion
280	5	Parasagittal	Lateral supramamillary region and zona incerta
286	3	Frontal	Lateral hypothalamus
287	3	Frontal	Zona incerta
289	3	Frontal	Medial and lateral hypothalamus
293	7	Parasagittal	Anterior mamillary region, medial hypothalamus and anterior thalamus
302	4	Parasagittal	Medial supramamillary region and zona incerta
310	5	Parasagittal	Medial hypothalamus and septum
330	4	Parasagittal	Midbrain
331	4	Parasagittal	Midbrain
346	5	Parasagittal	Midbrain
347	5	Parasagittal	Midbrain
350	5	Parasagittal	Mamillary bodies
353	5	Parasagittal	Anterior mamillary region, medial hypothalamus and anterior thalamus

PART I. THE MAMILLO-THALAMIC AND MAMILLO-TEGMENTAL DEGENERATION

Results

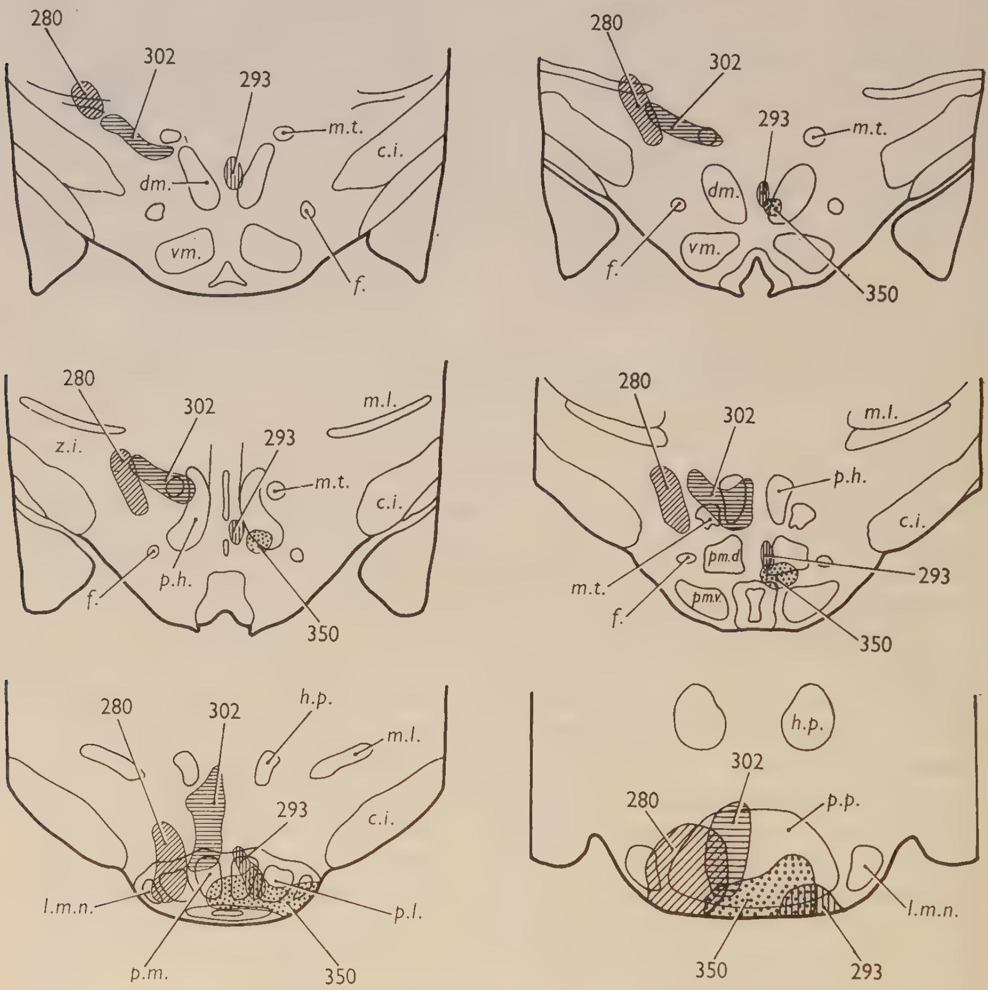
The lesions that include the mamillary bodies are shown in Text-fig. 1. Only the damage that is relevant to the mamillo-thalamic and mamillo-tegmental degeneration has been described in this section. Fuller descriptions of the non-mamillary damage are given in Part II. The mamillary bodies were damaged in *rats* 280, 293, 302, 350 and 353 but *rat* 353 is not shown in Text-fig. 1.

In *rat* 350 the lesion includes parts of all the medial mamillary elements and a small part of the lateral mamillary nucleus on the right. On the left only a small ventro-medial part of the medial mamillary nucleus has been damaged (Text-fig. 1). Beyond the boundaries of the mamillary region the lesion only includes a small portion of the posterior and medial hypothalamic periventricular region.

There is degeneration in both mamillo-thalamic tracts, denser on the right than on the left. On the right there is moderate to dense degeneration in all the anterior thalamic nuclei and on the left there is moderate to dense degeneration in the anterodorsal nucleus, the adjacent part of the anteroventral nucleus and the anterior and ventral parts of the anteromedial nucleus. Coarse degeneration granules run through the inter-anterodorsal commissure but the degeneration does not extend beyond the three anterior thalamic nuclei.

On the right heavy mamillo-tegmental degeneration can be traced into the midbrain. The plane of the sections is slightly oblique so that it has been possible to see almost the whole of the extent of the mamillo-tegmental tract on a single section. A scale drawing of this section is shown in text-fig. 2. The mamillo-tegmental fibres run caudally, medial to the habenulo-peduncular tract and medial to the red nucleus. In the midbrain they fan out in a dorsoventral direction so that some of the fibres lie immediately ventral to the medial longitudinal bundle while others lie further ventrally and pass through the tegmental decussations and the decussation of the superior cerebellar peduncle (Pl. 1, fig. 5).

Some of the most dorsal mamillo-tegmental fibres enter the central grey close to the midline and caudal to the nucleus of the fourth nerve, but this degeneration is only sparse in rat 350. Other fibres turn dorsally caudal to the decussation of the superior cerebellar peduncle. These run through and caudal to the deep tegmental nucleus of Gudden and a few reach the dorsal tegmental nucleus (Pl. 2, fig. 8) by



Text-fig. 1. The distribution of the lesions in *rats* 280, 293, 302, *and* 350. Note: the most posterior of the sections, passing through the pars posterior of the medial mamillary nucleus is shown at twice the magnification of the other sections.

passing through and lateral to the medial longitudinal bundle. Finally, another group of fibres turns caudo-ventrally from the caudal end of the decussation of the superior cerebellar peduncle to end in a dense plexus close to the midline, dorsal to the rostral part of the pontine nuclei (Text-fig. 2). This degeneration does not cross the midline and lies in an area that is clearly defined on the Nauta sections (Pl. 1, fig. 4), but not marked by any special characteristics on the Nissl sections. The

region in which these fibres end is the rostral and medial portion of the tegmental reticular nucleus of Bechterew (1899). On the left there is only sparse degeneration in the mamillo-tegmental tract. A few fibres could be followed as far as the caudal level of the red nucleus but were lost beyond this.

Text-fig. 2. The mamillo-tegmental and periventricular degeneration in rat 350. Parasagittal section. Note: (1) the smaller dots represent degeneration that lies on adjacent sections; (2) the deep tegmental nucleus lies lateral to this section but its relative position has been shown.

In *rat* 280 the lesion lies in the pars lateralis of the medial mamillary nucleus, in the lateral mamillary nucleus and in the lateral part of the supramamillary region. Homolaterally there is heavy degeneration in the mamillo-thalamic and mamillo-tegmental tracts. The fibres of the mamillo-thalamic tract traverse the antero-medial thalamic nucleus in small bundles and run dorsolaterally towards the antero-dorsal nucleus, where they form a coarse, dense plexus of degenerating fibres (Pl. 1, fig. 1). The anteroventral nucleus is practically free of degeneration and so are the parataenial, anterior paraventricular, rhomboid and reuniens nuclei.

A number of coarse degenerating fibres cross the midline in the inter-anterodorsal commissure. These run to the contralateral anterodorsal nucleus where they form a coarse plexus that is almost as dense as that in the homolateral nucleus (compare figs. 1 and 2 of Pl. 1). There is no other degeneration in the contralateral anterior thalamus.

The mamillo-tegmental tract lies among other fibres which are passing from the hypothalamus to the tegmentum in this animal (see Part II), and it cannot always be clearly distinguished from these. However, the characteristic mamillo-tegmental bundles run through the tegmental decussations and the decussation of the superior cerebellar peduncle as in rat 350. The whole of the central grey caudal to the fourth nerve nucleus, including the dorsal tegmental nucleus, is filled with dense degeneration but it is probable that a considerable part of this, in particular that lying in the lateral parts of the central grey, arises from the supramamillary rather than the mamillary part of the lesion (see Part II).

In *rat* 280 there is also a small lesion in the anterior and ventral parts of the pons, so that it has not been possible to trace the complete path of the caudo-ventral, reticular part of the mamillo-tegmental tract. A number of fibres run towards the tegmental reticular nucleus from the caudal end of the superior cerebellar peduncle, but these are lost among other degenerating fibres at the rostral levels of the pons.

On the contralateral side there are a few granules in the mamillo-tegmental tract but these could not be followed far beyond the habenulo-peduncular tracts. There is no clear evidence for any considerable crossing of the mamillo-tegmental tracts such as has been described by Sanz (1935).

In *rat* 302 the lesion lies in the bifurcation of the principal mamillary tract and degeneration can again be traced into each of the three homolateral anterior thalamic nuclei and to the contralateral anterodorsal thalamic nucleus. The mamillo-tegmental tract can be traced to the central grey caudal to the fourth nerve nucleus, including the medial parts of the dorsal tegmental nucleus (Pl. 2, fig. 7), and to the tegmental reticular nucleus. In this animal a few fibres also pass further ventrally into the anterior parts of the pontine nuclei.

In *rat* 293 the lesion passes through the anterior and medial parts of the medial mamillary nucleus on the right, including the pars medianus, the anterior and ventral part of the pars medialis and a very small part of the pars posterior. Rostrally the lesion passes through the hypothalamus close to the midline, through the medial part of the nucleus reuniens on the left and the lesion ends in the left parataenial nucleus.

On the right there is dense degeneration in the anterior part of the anteromedial and the adjacent part of the anteroventral thalamic nucleus. The degeneration is sparser in the other parts of these nuclei and there is no degeneration in the anterodorsal nucleus. On the left there is no anterior thalamic degeneration apart from some granules which come from the thalamic part of the lesion and enter the parataenial and paraventricular nuclei. Sparser degeneration, which probably represents the medial part of 'component B' of the fornix (Guillery, 1956), also enters the anteromedial thalamic nucleus on the left. The distribution of the mamillo-tegmental tract is almost identical to that found in *rat* 350.

In *rat* 353 the lesion is similar to that found in *rat* 293 but the mamillary part of the lesion lies further rostrally so that only a very small ventral part of the pars medialis and medianus has been damaged. There is only sparse degeneration in the two efferent mamillary tracts and neither could be followed far. The mamillo-thalamic degeneration does not extend beyond the anteromedial nucleus.

These five animals thus support the view that the mamillo-thalamic tract distributes to each of the three anterior thalamic nuclei and to the contralateral anterodorsal thalamic nucleus and that there is some topographical organization in the mamillo-thalamic projection. They show that the mamillo-tegmental tract passes to the tegmental reticular nucleus and to the central grey caudal to the fourth nerve nucleus.

DISCUSSION

Le Gros Clark (1933) and Powell & Cowan (1954) have previously studied the mamillo-thalamic projection in the rat. The former used the Marchi method and the latter used the method of retrograde cell degeneration. Le Gros Clark showed that the mamillo-thalamic tract ends in each of the three homolateral anterior thalamic nuclei and that a number of fibres cross in the interanterodorsal commissure to end the contralateral anterodorsal nucleus. Yamagata (1927) found a similar pattern of degeneration in the rabbit. The present series of animals confirms these findings and shows that the anterodorsal nucleus is the only one of the three anterior thalamic nuclei to receive a crossed projection.

Powell & Cowan showed that each of the elements in the medial mamillary nucleus has an independent projection to one of the anterior thalamic nuclei; the pars medialis projects to the anteromedial nucleus, the pars posterior to the anteroventral nucleus and the pars lateralis to the anterodorsal nucleus. The lateral mamillary nucleus shows no retrograde changes after anterior thalamic lesions.

Rat 280 agrees with these findings in showing that either the pars lateralis or the lateral mamillary nucleus projects to the anterodorsal thalamic nucleus, but *rat* 293 appears to contradict these findings, since there is dense degeneration in the ventromedial parts of the anteroventral nucleus which cannot be accounted for by the small amount of damage to the pars posterior of the medial mamillary nucleus. The degeneration in the anteroventral nucleus does not come from the thalamic part of the lesion since it can be traced into the mamillo-thalamic tract and *rat* 353, in which there was a similar anterior thalamic lesion but less danage to the mamillary region, showed no degeneration in the anteroventral thalamic nucleus. Cajal (1911) has described the initial course of the efferent mamillary axons as 'irregular and variable'. The method used in the present investigation would only show the precise topographical organization of the mamillo-thalamic pathway if the fibres ran a direct course from their cells of origin to the mamillo-thalamic tract. It seems probable that a number of fibres from the pars posterior pass through the pars medialis on their way to the mamillo-thalamic tract. Alternatively, the fibres that ramify in the anteromedial thalamic nucleus may send collaterals to the adjacent parts of the anteroventral nucleus but there is no evidence for this. In either case, since the initial segments of the mamillo-thalamic axons send collaterals to the mamillary cells (Cajal, 1911) there is a possibility for interaction between the three separate mamillo-thalamic projections.

Powell & Cowan found that the pars lateralis of the medial mamillary nucleus undergoes extensive retrograde degeneration after unilateral lesions in the anterodorsal thalamic nucleus. The appearances that they describe would not be expected if a large number of collaterals passing to the contralateral anterodorsal thalamic nucleus from the pars lateralis had remained intact. It is more likely that the pars

lateralis of the medial mamillary nucleus has a unilateral projection to the anterodorsal thalamic nucleus and that the lateral mamillary nucleus, which does not suffer retrograde changes after unilateral thalamic lesions, has a bilateral projection to the anterodorsal nucleus. This would agree with the relatively coarse degeneration that occurs in the anterodorsal nucleus for it is known that the efferent fibres from the lateral mamillary nucleus are coarser than those from the medial mamillary nucleus (Tello, 1936; Guillery, 1955) and Tello has also shown that a number of these coarser fibres pass into the mamillo-thalamic tract. Such an interpretation would appear to be in contradiction to the experiment of van Valkenberg (1911–12) who found cell degeneration in the lateral mamillary nucleus after a midbrain lesion. However, his lesion was very close to the lateral mamillary nucleus itself, so that this may not have been a critical experiment as regards the projection of the lateral mamillary nucleus.

Comparative studies further suggest that there may be a link between the lateral mamillary nucleus and the anterodorsal thalamic nucleus. The lateral mamillary nucleus receives the great majority of its afferent fibres from the mamillary peduncle and the anterodorsal nucleus sends its efferent fibres to the retrosplenial cortex. The mamillary peduncle, anterodorsal nucleus and retrosplenial cortex are relatively much larger in the rabbit and the rat than they are in the monkey and in man. The cat occupies an intermediate position (Rose & Woolsey, 1948; Powell, Guillery & Cowan, 1956).

The mamillo-tegmental tract has been studied by a large number of observers. Gudden (1889) first described its ending as among the cells of the deep tegmental nucleus and a similar ending has been described by Koelliker (1896) and Morin (1950). Probst (1900) described a number of mamillo-tegmental fibres which turn ventrally caudal to the level of the fourth nerve nucleus into the substantia reticularis of the same side. Economo & Karplus (1910) and Sanz (1935) were able to follow the mamillo-tegmental tract as far as the deep tegmental nucleus and report that a number of fibres also pass through this nucleus into the reticular formation of the pons and bulb. Yamagata (1927) reported degeneration in the deep and dorsal tegmental nuclei after a lesion in the medial mamillary nucleus.

In the present material mamillo-tegmental degeneration has been traced to the deep tegmental nucleus, but it has never been possible to see an extensive pericellular plexus in this nucleus. The mamillo-tegmental fibres appear largely to pass through this nucleus; a few may end there. A group of fibres passing ventral to the deep tegmental nucleus to the anteromedial parts of the tegmental reticular nucleus has been found in all the animals. Variable degeneration has also been found in the dorsal tegmental nucleus and adjacent central grey. It may be significant that the dorsal tegmental degeneration is heaviest in the animals in which the efferent fibres from the lateral mamillary nucleus have been damaged, but the precise organization of the projection from the lateral mamillary nucleus and the origin of the mamillo-tegmental tract need further investigation (see also above). In the present material it has never been possible to trace any of the mamillo-tegmental degeneration to levels caudal to the genu of the seventh cranial nerve.

Brodal (1954) has included the tegmental reticular nucleus among the pontine nuclei and has shown that it sends its axons to the cerebellum. Walberg (1956) has

found a projection from the periaqueductal grey to the inferior olive. Both observations suggest that there is a close relationship between the mamillary bodies and the cerebellum. In view of this it appears worth re-investigating the hypothalamo- or mamillo- cerebellar connexions that have been described in Amphibia from time to time (see, for example, Larsell, 1931). It is also of interest to note that Cajal (1911) described the fibre endings in the dorsal tegmental nucleus as similar to the ending of the mossy fibres in the cerebellum. According to Cajal, however, these fibres come from the interpeduncular nucleus, not from the mamillo-tegmental tract.

It has not been possible to see degeneration in the interpeduncular nucleus in any of the animals described above. Although there are a number of degenerating fibres passing caudally dorsal to the mamillary peduncle there are only very few degenerating fibres in the mamillary peduncle itself. It must be concluded that there are no mamillo-peduncular fibres in the rat and that the only fibre group which passes caudally from the mamillary bodies is the mamillo-tegmental tract.

PART II. THE MEDIAL FOREBRAIN BUNDLE, PERIVENTRICULAR SYSTEM AND INTRAHYPOTHALAMIC CONNEXIONS

Results

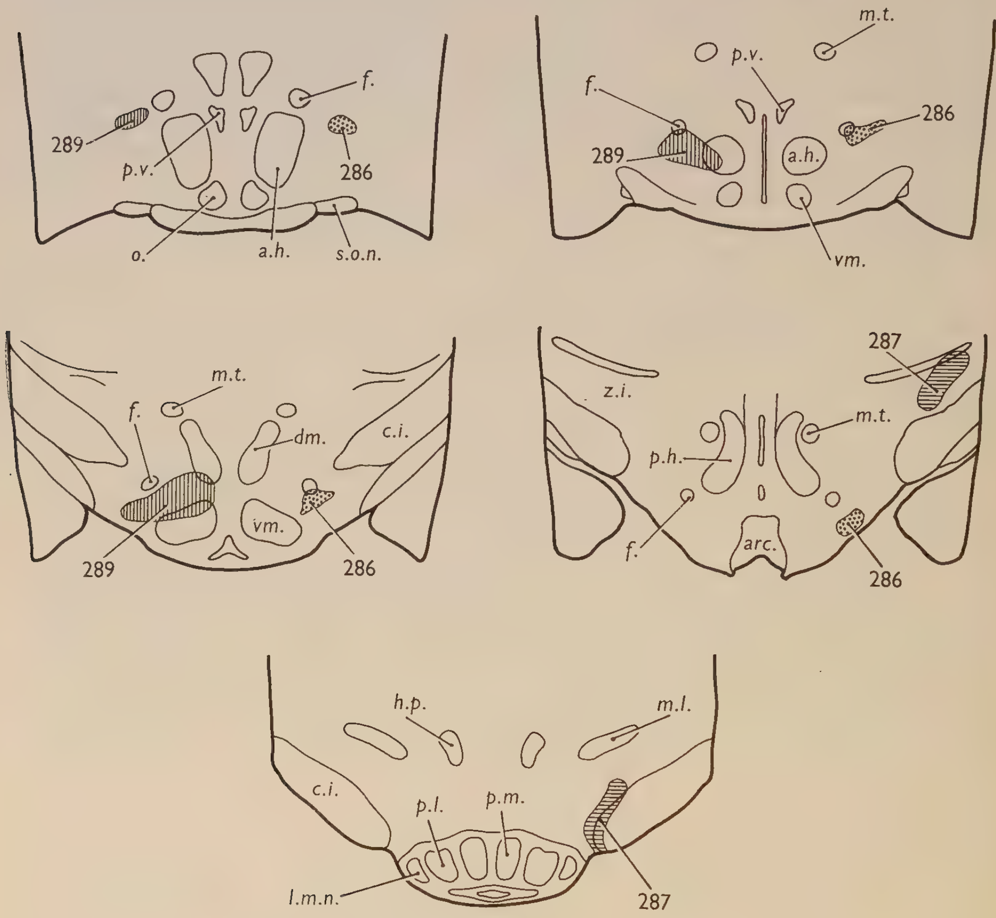
The fibres of the medial forebrain bundle lie scattered throughout the lateral hypothalamic region ventral and lateral to the post-commissural fornix. Rostrally the fibres run through the lateral preoptic region and turn dorsomedially into the septum. Caudally the fibres divide into two main groups at the level of the mamillary bodies. One group continues caudally into the tegmental region of the midbrain, running just dorsal to the mamillary peduncle, the other turns dorsomedially into the central grey substance of the midbrain, where it joins the periventricular fibres. The term 'medial forebrain bundle' will not be confined to the hypothalamic course of these fibres, but will be applied to the whole of the course of the fibres that form a part of the bundle in the lateral hypothalamic region. The bundle is made up of ascending and descending fibres which appear to be mingled throughout most of its course. The ascending fibres are described first.

Ascending degeneration in the medial forebrain bundle

The ascending degeneration in the medial forebrain bundle is shown clearly in *rat* 289 in which the lesion lies in the lateral hypothalamus, extending from the level of the ventromedial nucleus to the level of the nucleus ovoideus. The lesion includes the ventrolateral part of the fornix and extends medially into the adjacent parts of the ventromedial and dorsomedial hypothalamic nuclei. It does not cross the midline (Text-fig. 3).

There is heavy degeneration in the homolateral medial forebrain bundle and this can be followed through the lateral preoptic region to the level of the anterior commissure. Here the degenerating fibres turn first medially, filling the whole of the subcommissural region with degeneration products, and then dorsally into the septum as shown in Text-fig. 4. The septal degeneration can be roughly divided into two groups. A sparse, medial group of relatively coarse fibres occupies the medial septal nucleus and the nucleus of the diagonal band. A denser, lateral group of

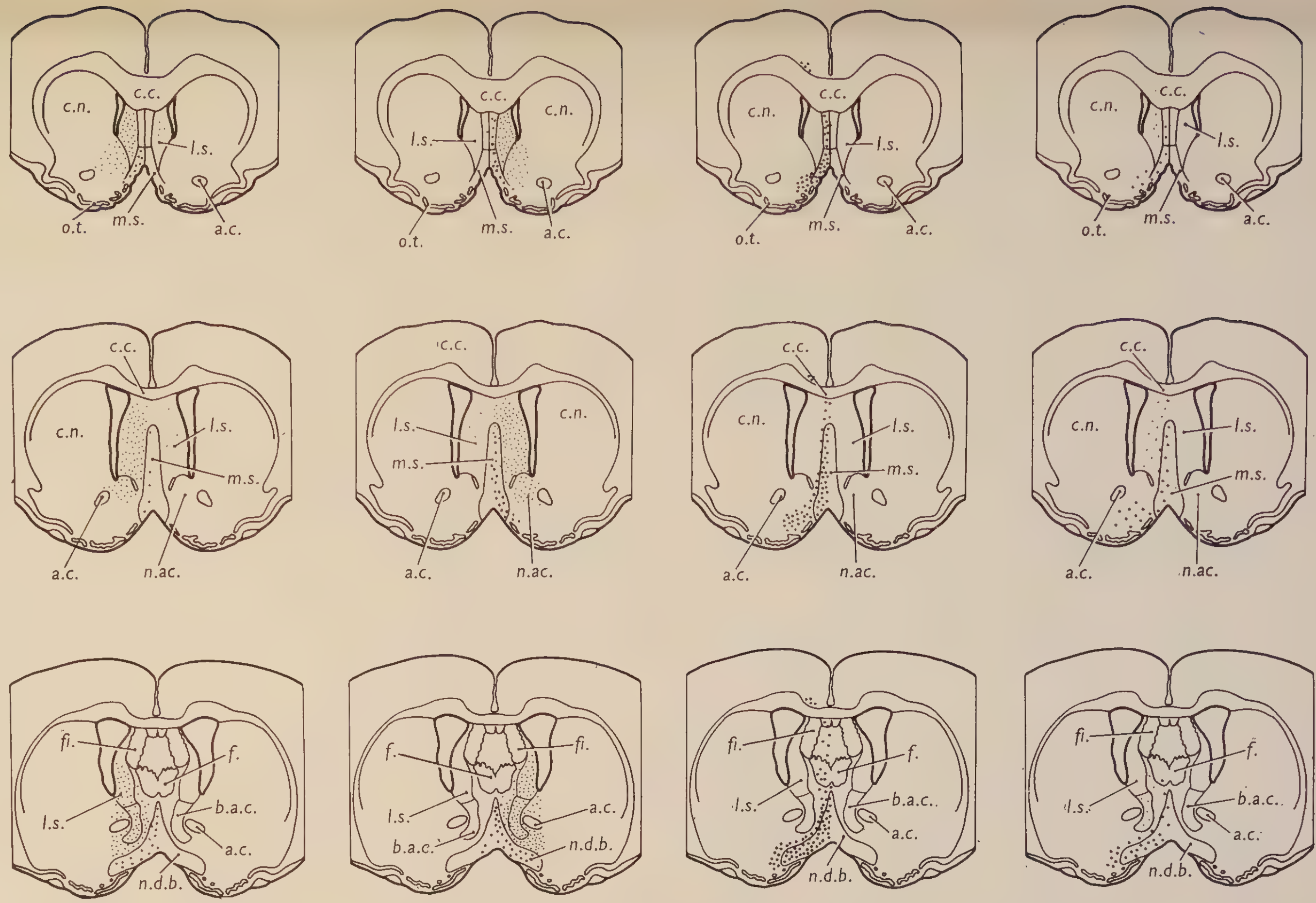
relatively fine fibres occupies most of the lateral septal nucleus and the dorsal part of the nucleus accumbens. Posteriorly these fibres also lie in the bed nucleus of the anterior commissure. In the following account the first group will be called the medial and the second the lateral component of the medial forebrain bundle. This refers to their septal relationships and not to their relative positions in the hypothalamic part of the medial forebrain bundle.



Text-fig. 3. The distribution of the lesions in rats 286, 287 and 289.

On the contralateral side there is only a small island of degeneration in the lateral septal nucleus. Since a number of fibres cross in the supraoptic commissures it is possible that a few fibres reach the contralateral medial forebrain bundle by this route, but a crossing within the septum is also possible.

In *rat* 286 the lesion is confined to the lateral hypothalamus, extending from the premamillary region to the level of the nucleus ovoideus. The ventrolateral part of the fornix has again been damaged (Text-fig. 3). The ascending degeneration in the medial forebrain bundle is similar to that described for *rat* 289 except that the lateral septal degeneration is slightly denser and the medial septal degeneration



Rat 289 Rat 286 Rat 280 Rat 302

Text-fig. 4. Frontal sections through the telencephalon to show the distribution of the septal degeneration after varying hypothalamic lesions. Rat 289: lesion in the medial and lateral hypothalamus. Rat 286: lesion in the lateral hypothalamus only. Rat 280: lesion in the lateral supra-mamillary region. Rat 302: lesion in the medial supra-mamillary region and posterior hypothalamus.

decidedly denser than in that animal (Text-fig. 4, and compare figs. 9 and 10 in Pl. 2). The contralateral septum shows only sparse degeneration in the lateral septal nucleus and nucleus accumbens. Again, there are some degenerating fibres crossing in the supraoptic commissures but these could not be traced into the contralateral medial forebrain bundle.

In *rat* 280 the medial component of the medial forebrain bundle shows heavy degeneration but the lateral component is normal. The lesion (Text-fig. 1) passes through the mamillary region and into the lateral part of the supramamillary region. It then passes rostrally, laterally and dorsally through the zona incerta to end in the medial part of the medial lemniscus as this enters the thalamus.

From the supramamillary part of the lesion a dense group of degenerating fibres can be traced rostrally through the lateral hypothalamus and lateral preoptic region. The density of the degeneration does not decrease markedly as the bundle is followed rostrally, but there may well be fibre endings around the cells of the lateral hypothalamus. At the level of the anterior commissure the fibres turn medially and dorsally and fill the diagonal band and medial septal nucleus with dense degeneration (Text-fig. 4). Some of the fibres lie in the ventral part of the nucleus accumbens, but the dorsal part of the nucleus accumbens, the lateral septal nucleus and the bed nucleus of the anterior commissure are free of degeneration granules. From the hypothalamic part of the medial forebrain bundle a number of fibres can be seen passing towards the supraoptic commissures but there are only a few granules in the contralateral medial septal nucleus and none in the contralateral lateral septal nucleus.

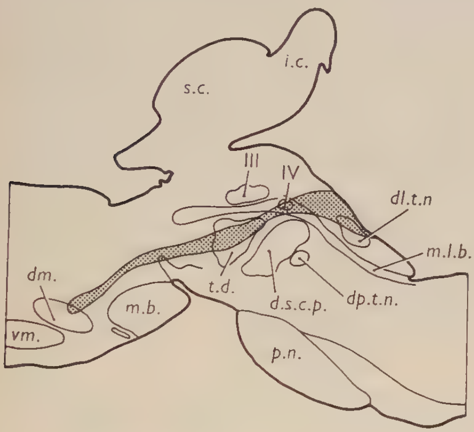
Some of the degeneration in the medial septal nucleus can be traced towards the genu of the corpus callosum rostrally and towards the dorsal fornix caudally. There are a few degenerating fibres in the supracallosal striae and a few in the dorsal fornix. On the more medial sections of the series there is very sparse degeneration in the stratum radiatum and the stratum lacunosum of the cornu ammonis and there is sparse degeneration in the medial parts of the subiculum.

Rat 280 shows that the medial component of the medial forebrain bundle passes through the lateral part of the supramamillary region and it suggests that the lateral component arises at more rostral levels. The degeneration that passes from the zona incerta to the hypothalamus will be discussed in a separate part of this paper, but it is relevant here that there are few if any fibres passing from the zona incerta to the medial forebrain bundle. This is shown by *rat* 287 in which the lesion includes a large part of the zona incerta but spares all except a very small lateral portion of the supramamillary region (Text-fig. 3). This animal shows practically no ascending degeneration in the medial forebrain bundle.

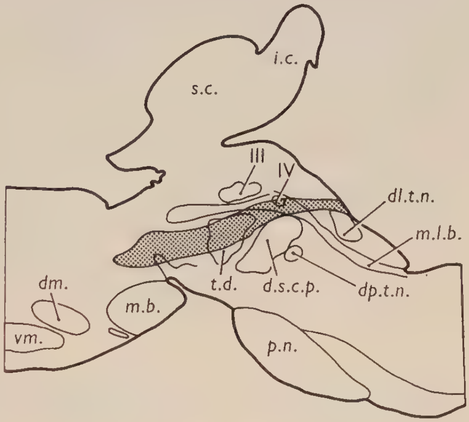
In *rats* 293 *and* 302 the lesion passes through the medial parts of the medial mamillary nucleus and through the supramamillary region medial to the principal mamillary tract (Text-fig. 1). In *rat* 293 the rostral part of the lesion passes through the periventricular part of the right hypothalamus, lies in the midline between the paraventricular nuclei, passes through the medial part of the nucleus reuniens on the left side of the midline and ends in the ventral part of the left parataenial nucleus. In *rat* 302 the rostral part of the lesion deviates dorsolaterally into the medial part of the zona incerta. There is only moderate to sparse ascending degeneration in the

medial forebrain bundles of these two animals, much sparser than that described previously. Text-fig. 4 shows the distribution of the septal degeneration in *rat* 302; there is moderate to sparse degeneration in the medial component of the medial forebrain bundle and very sparse degeneration in the lateral component. In *rat* 293 there is moderate to sparse ascending degeneration in the right medial forebrain bundle, running from the region anterolateral to the mamillary bodies to the diagonal band. There is sparse, fine, bilateral degeneration in the bed nucleus of the anterior commissure, but there are only a few scattered, doubtful granules in the rest of the septum.

These two animals confirm that the greater part of the medial component of the medial forebrain bundle passes through the lateral supramamillary region, the principal mamillary tract probably marking the medial boundary of the component. The lateral component of the medial forebrain bundle must arise in the premamillary hypothalamus.



Text-fig. 5. The lesion in *rat* 330.



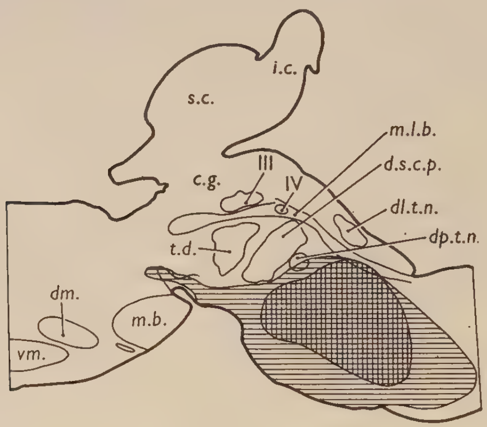
Text-fig. 6. The lesion in *rat* 331.

Four lesions have been placed in the mid-brain in an attempt to show whether or not the medial component arises caudal to the hypothalamus. The approximate extent of these lesions is shown in Text-figs. 5–7. In *rat* 330 the lesion lies entirely on the left side, entering the central grey caudal to the fourth nerve nucleus, damaging the dorsal tegmental nucleus, the fourth nerve nucleus, the tegmental decussations, the medial part of the habenulo-peduncular tract, the medial supramamillary region and the posterior part of the dorsomedial hypothalamic nucleus. The lesion lies close to the midline and does not extend laterally beyond the red nucleus or habenulo-peduncular tract.

There is degeneration in the mamillary peduncle on the side of the lesion and a few fibres may pass from this into the medial forebrain bundle (see Guillery, 1956). Other fibres run from the region close to the habenulo-peduncular tract to the supramamillary region, turn ventrally and laterally lateral to the principal mamillary tract and join the hypothalamic medial forebrain bundle in the posterior part of the lateral hypothalamus. There are not many of these fibres, but moderate degeneration can be traced through the lateral hypothalamus and into the diagonal band.

Most of the degeneration stops in the diagonal band; only a few scattered granules can be seen in the medial septal nucleus or the nucleus accumbens. A number of fibres cross the midline in the supramamillary region, but there is only doubtful degeneration in the contralateral medial forebrain bundle.

In *rat* 331 the lesion is similar to that found in *rat* 330 (compare Text-figs. 5 and 6). It includes the tegmental decussations, the posterior and medial part of the red nucleus, the habenulo-peduncular tract and the medial part of the supramamillary region on the right. The dorsal tegmental nucleus on the right has been spared but that on the left has been damaged. There is degeneration in the mamillary peduncle on the left but not on the right and again, some of the mamillary peduncle fibres can be traced into the medial forebrain bundle. On the right the degeneration in the medial forebrain bundle is similar to that found in rat 330.



Text-fig. 7. The lesion in *rat* 346. Horizontal lines show the extent of the lesion on the left, vertical lines the extent on the right.

In *rats* 346 *and* 347 a large part of the ventral pons and midbrain has been damaged (Text-fig. 7). In each of these animals there is coarse, dense, ascending bilateral degeneration in the medial component of the medial forebrain bundle but none in the lateral component. In *rat* 346 more of the lesion lies on the left. On the right the lesion does not extend laterally beyond the level of the deep tegmental nucleus and lies just posterior to this. On the left the lesion extends laterally as far as the exit of the fifth nerve and anteriorly as far as the interpeduncular nucleus, including the ventral part of the habenulo-peduncular tract, the mamillary peduncle and the region dorsal to the mamillary peduncle.

On the right degenerating fibres pass anteriorly through the rostral midbrain a short distance dorsal to the mamillary peduncle.These fibres appear to have passed through or ventral to the tegmental decussations. They enter the supramamillary region by passing on either side of the habenulo-peduncular tract, more running lateral than medial to this tract. Within the supramamillary region these fibres run ventrolaterally towards the posterior part of the lateral hypothalamus as in *rats* 330 *and* 331. On the left side the supramamillary part of the medial forebrain

bundle can be seen arising from the most rostral part of the lesion. The rostral distribution of the medial forebrain bundle is practically identical to, but slightly sparser than, that found in *rat* 280. There is again some sparse, scattered degeneration in the medial part of the cornu ammonis and subiculum. There are also a number of degenerating fibres in the medial parts of the supramamillary region which do not enter the medial forebrain bundle. Some pass into the most posterior parts of the premamillary hypothalamus (see below) and others form a part of the supramamillary commissure.

The ascending fibres in the medial forebrain bundle can thus be divided into two distinct groups. One group arises in the premamillary hypothalamus, travels through the lateral hypothalamus and preoptic region and passes below the anterior commissure into the bed nucleus of the anterior commissure, the lateral septal nucleus and the dorsal part of the nucleus accumbens. It is a hypothalamo-septal bundle. The other arises in the ventral part of the mid-brain or pons. A part of this second group must arise from a region posterior to the decussation of the superior cerebellar peduncle, but the precise origin is not clear at present. These fibres pass through the supramamillary region lateral to the principal mamillary tract, through the lateral hypothalamus and lateral preoptic region and into the diagonal band and medial septal nucleus. Some of these fibres pass into the ventral part of the nucleus accumbens and some may enter the hippocampal formation. Most of the fibres in this group probably form a mesencephalo-septal pathway. However, there may be additional cells of origin in the pons, and there may be additional endings in the lateral hypothalamus and hippocampus.

Descending degeneration in the periventricular system and medial forebrain bundle

In *rat* 350 the medial forebrain bundle has been completely spared. A small part of the periventricular system of the caudal hypothalamus has been damaged, and Text-fig. 2 shows the periventricular degeneration passing dorsally and caudally into the mesencephalic central grey (see also Pl. 1, fig. 3). On the right (homolaterally) there is moderate to dense degeneration in the dorsal and lateral parts of the central grey rostral to the level of the third nerve nucleus. On the left there is sparse degeneration in the more ventral and rostral parts of the central grey, and this can be traced rostrally towards the supramamillary region. These two fibre groups, running from the hypothalamus to the mesencephalic central grey have been found in a number of animals. In the following descriptions the first group, which passes into the dorsal parts of the central grey and runs medial to the principal mamillary tract, will be called the rostral periventricular component; the second group, which passes through the supramamillary region and into the ventral parts of the central grey, will be called the caudal periventricular component. Neither of these fibre groups forms a terminal plexus in relation to any particular periaqueductal cell group. The degeneration runs antero-posteriorly through the periaqueductal grey and gradually becomes sparser as it approaches the level of the third nerve nucleus.

In *rats* 293 *and* 353 the lesion again lies medially, sparing the hypothalamic part of the medial forebrain bundle but damaging the periventricular fibres (Text-fig. 1). There are a number of fibres passing into the homolateral rostral component and a

few cross in the supramamillary region to enter the caudal periventricular component of the opposite side.

In *rat* 302 the lesion extends further laterally (Text-fig. 1). From the dorsomedial part of the lesion dense degeneration passes caudally in the rostral periventricular component and sparse to moderate degeneration enters the caudal periventricular component. The majority of the fibres in the latter group are crossed but there are also a few uncrossed fibres entering the caudal component.

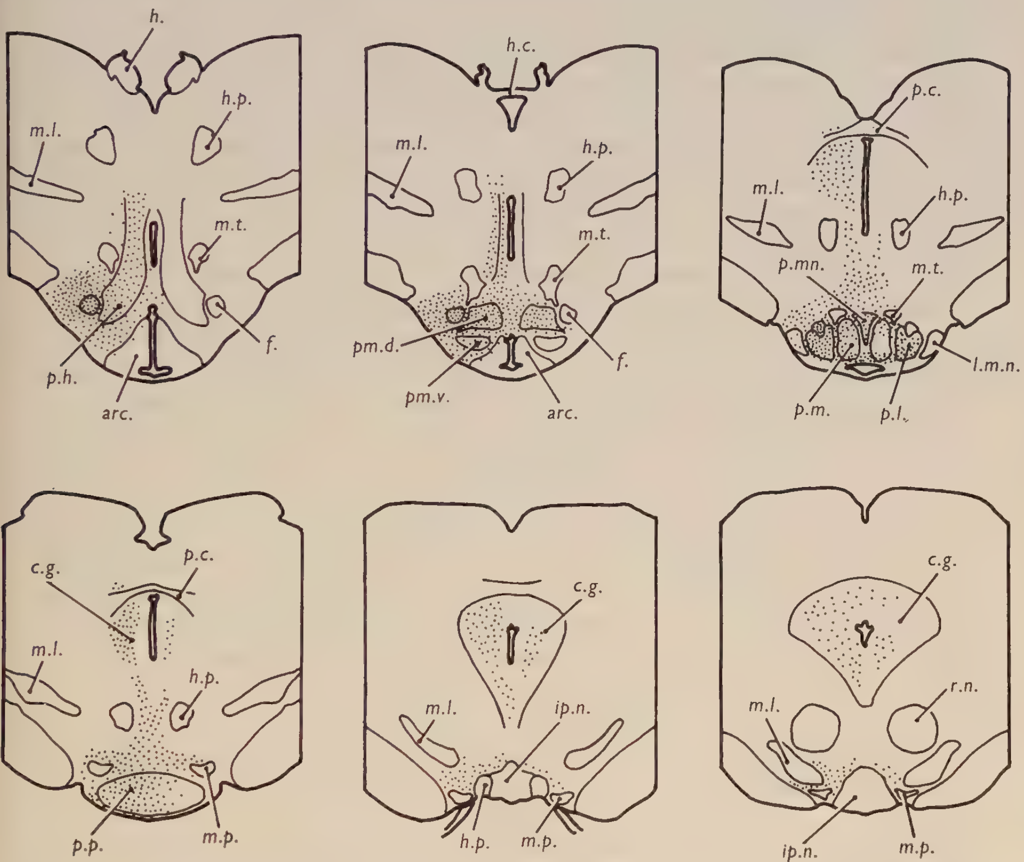
A small medial part of the medial forebrain bundle has been damaged in this animal and sparse to moderate degeneration can be seen running caudally through the supramamillary region and into the ventromedial parts of the midbrain tegmentum, where it lies dorsal to the mamillary peduncle, dorsolateral to the interpeduncular nucleus and dorsomedial to the medial lemniscus. This degeneration has not been traced into a definite terminal plexus. It becomes sparser as it is followed caudally and at the level of the decussation of the superior cerebellar peduncle most has disappeared. A few fibres can be seen turning dorsolaterally caudal to the red nucleus and a few may join the ventral parts of the mamillo-tegmental tract but it has not been possible to trace any of these fibres far. Finally a small group of fibres passes from the lesion through the supramamillary commissure and then turns caudally dorsal to the contralateral mamillary peduncle.

In *rat* 280 the lesion passes through the lateral part of the supramamillary region but there is also a small lesion in the rostral part of the pons. In the following description only the degeneration that can be traced clearly to the supramamillary lesion will be described. There is a small group of degenerating fibres in the rostral periventricular component; this arises in the lateral supramamillary region and passes dorsomedially and anteriorly into the posterior hypothalamus before it again turns posteriorly into the rostral periventricular component. There is also moderate degeneration passing from the supramamillary region into the caudal periventricular component. This degeneration is bilateral and extends as far as the third nerve nucleus in the ventral parts of the central grey. It does not enter the nucleus of the third nerve.

There is dense degeneration passing directly caudally into the ventromedial parts of the midbrain tegmentum. A large number of these fibres turn dorsolaterally at the caudal level of the red nucleus, and many can be followed between the red nucleus and the decussation of the superior cerebellar peduncle, running towards the lateral parts of the central grey. They enter the central grey as a scattered group lateral to the medial longitudinal bundle at the level of and caudal to the nucleus of the fourth nerve. A number run into the most lateral part of the central grey where they come into close relation with the mesencephalic nucleus of the fifth nerve. Other fibres do not turn dorsolaterally in this way but continue caudally ventral to the decussation of the superior cerebellar peduncle and then turn dorsally towards the dorsal tegmental nucleus. They lie in close relation to the ventral fibres of the mamillo-tegmental tract and cannot be clearly distinguished in the present material.

There is sparse degeneration dorsal to the contralateral mamillary peduncle. Rostrally this is continuous with degeneration in the supramamillary commissure and caudally it cannot be followed beyond the decussation of the superior cerebellar peduncle.

In *rats* 289 *and* 286 only the premamillary hypothalamus has been damaged but each animal shows considerable degeneration in the midbrain. In *rat* 289, where the lesion included parts of the medial and lateral hypothalamus, the whole of the posterior hypothalamus on the side of the lesion is packed with heavy degeneration. (Text-fig. 8). From the posterior hypothalamus a dense group of degenerating fibres turns dorsomedially into the rostral periventricular component. A number of these fibres appear to come from the medial part of the medial forebrain bundle but



Text-fig. 8. The caudal degeneration after a lesion in the medial and lateral hypothalamus. Rat 289.

others may come directly from the medial hypothalamus. The supramamillary region is also filled with dense degeneration. From this fibres pass into the caudal periventricular component of both sides, other fibres continue in the ventromedial parts of the homolateral midbrain and others cross in the supramamillary commissure and then run caudally dorsal to the contralateral mamillary peduncle (Text-fig. 8). None of the fibres could be followed clearly beyond the level of the third nerve nucleus on this frontal series. Some of the ventromedial mesencephalic fibres could be seen turning dorsolaterally just rostral to the decussation of the superior cerebellar peduncle but these fibres could not be traced far. Caudal to the

level of the third nerve nucleus there is doubtful degeneration in the lateral parts of the central grey. This extends caudally as far as the laterodorsal nucleus (Gillilan, 1943), and is heavier on the homolateral side than it is on the contralateral side. However, the plane of the sections, perpendicular to these fine fibres, makes it difficult to identify this as definite degeneration.

Rat 286 in which the lesion damaged the lateral hypothalamus but spared the medial hypothalamus shows a pattern of degeneration similar to the above. However, the degeneration in the rostral periventricular component is only sparse, suggesting that the greater part of this comes from the medial hypothalamus.

Finally, *rat* 310 in which the lesion passes through the most medial part of the premamillary hypothalamus on the left and then destroys the greater part of the septum and fornix on the right shows some degeneration in the midbrain. On the left there is moderate to sparse degeneration in the rostral periventricular component. On the right there is sparse degeneration in the caudal periventricular component and sparse degeneration dorsal to the mamillary peduncle. The last may well be due to the degeneration in the post-commissural fornix.

The efferent hypothalamo-mesencephalic fibres thus fall into two groups, one passing into the periaqueductal grey and the other into the ventro-medial parts of the midbrain tegmentum. The latter passes through the ventral tegmental area of Tsai and through the ventral parts of the nucleus mesencephalicus profundus pars lateralis of Gillilan (1943). Some of the fibres may end among these cell groups, but others pass dorsolaterally and a number enter the central grey caudal to the fourth nerve nucleus. This group only degenerates after lesions that include the hypothalamic part of the medial forebrain bundle and must be regarded as a part of this bundle. Some of the fibres that enter the periaqueductal grey also run through the medial forebrain bundle, but others, particularly those running to the dorsal and lateral parts of the central grey pass directly from the medial hypothalamus to the central grey. The majority of the hypothalamo-mesencephalic fibres are uncrossed, but a number also cross the midline dorsal to the mamillary bodies.

Intrahypothalamic connexions

The present material does not provide detailed information about the intrahypothalamic connexions of the rat but a few points of interest have arisen. In *rat* 280 in which the lesion includes the lateral supramamillary region the medial hypothalamus is completely free of degeneration. In *rats* 302, 330 *and* 331, in which the more medial parts of the supramamillary region have been damaged, there is only moderate to sparse degeneration passing into the posterior parts of the dorsomedial hypothalamic nucleus. None reaches the ventromedial nucleus or the anterior hypothalamic nucleus but some passes into the dorsal hypothalamic region close to the zona incerta.

The medial hypothalamus thus receives only very few direct ascending fibres but *rat* 286, in which the lateral hypothalamus has been damaged and the medial hypothalamus spared, shows that all the medial hypothalamic nuclei receive cross connexions from the lateral hypothalamic area. In this way it may be possible for ascending impulses to reach the medial hypothalamus via a synapse in the lateral hypothalamus. The medial hypothalamus also receives a large number of direct

descending fibres. This is shown by *rat* 310 in which a large part of the septum has been damaged and in which there is heavy degeneration throughout the medial hypothalamus. However, the precise origin of these descending hypothalamic fibres cannot be determined from this single large lesion.

In *rats* 286 *and* 289 the post-commissural fornix has been damaged in the hypothalamic part of its course. The degenerating fornix fibres can be traced to the mamillary region, where they distribute to the pars lateralis and the pars posterior of the medial mamillary nucleus (Guillery, 1956). In *rats* 286 *and* 289 there is additional dense degeneration in the dorsal premamillary nucleus (Text-fig. 8). This extends across the midline to the opposite dorsal premamillary nucleus and also passes into the pars medianus and the anterior part of the pars medialis of the medial mamillary nucleus. This degeneration is much heavier than any that has been seen in this region after fornix lesions, and it must be assumed that a number of fibres pass from the premamillary hypothalamus into the anterior part of the medial mamillary nucleus.

A number of workers have also desribed incerto-hypothalamic fibres (see Gurdjian, 1927; Le Gros Clark, 1938). *Rats* 280, 287 *and* 302, in which a large part of the zona incerta has been damaged, provide no evidence for any considerable connexion between the zona incerta and the premamillary hypothalamus. In these animals only a few fibres enter the dorsal and lateral parts of the hypothalamus from the zona incerta.

Discussion

Descending connexions from the hypothalamus

Descending fibres which enter the midbrain via the hypothalamus have been described by a number of observers. Bischoff (1900) described a bundle, the olfacto-mesencephalic fasciculus, on the basis of Marchi degeneration in the hedgehog. The fibres of this bundle run through the lateral hypothalamus and reach the level of the red nucleus in the tegmentum. Wallenberg (1902) studied this bundle in the rabbit and found a pattern of mesencephalic degeneration which was in many respects similar to that reported here. He found a crossed and a direct projection to the rostral parts of the mesencephalic central grey and also traced a large number of fibres into the ventromedial parts of the midbrain tegmentum. Many of the latter were lost dorsolateral to the medial lemniscus at caudal midbrain or rostral pontine levels, but others entered the central grey at the level of the fourth nerve nucleus, or caudally, and some of these came into immediate medial relation to the mesencephalic nucleus of the fifth nerve. Wallenberg regarded the subcommissural region as the origin for the majority of these fibres and was able to trace a number of direct and crossed fibres as far as the cervical cord. However, his lesions were made by a dorsal approach and included parts of the cortex and internal capsule, so that the cervical degeneration may have travelled in the medial parts of the internal capsule. Bischoff's olfacto-mesencephalic tract arises rostral to the hypothalamus, but it seems probable from the present material that the majority of the fibres that enter the midbrain via the hypothalamus arise in the hypothalamus or preoptic region and form the main caudal outflow of the hypothalamus.

Beattie *et al.* (1930) made lesions caudal to the mamillary bodies using a ventral approach in cats. They found descending degeneration mainly in the central grey with only scattered degeneration in the reticular formation. However, their lesions included the medial supramamillary region but spared most of the lateral supramamillary region so that they interrupted the periventricular fibres but spared most of the others. Beattie *et al.* also describe fibres that descend as far as the lumbar cord. Their lesions may well have damaged the rostral part of the medial longitudinal bundle so that it is not certain that these long fibres form a part of the hypothalamic outflow. On the present material it has not been possible to trace hypothalamic degeneration beyond the level of the seventh nerve, and at present there is no good evidence to show that hypothalamic fibres pass further caudally. It is most probable that efferent impulses from the hypothalamus pass through at least one synapse before they reach the spinal cord.

Morin (1950) described descending degeneration passing from the medial hypothalamus to the periventricular system and from the lateral hypothalamus to the lateral part of the nucleus mesencephalicus profundus. This is in close agreement with the present results, except that in *rats* 286 *and* 280 it has been possible to see that a number of fibres also pass from the lateral hypothalamus to the periventricular system. Magoun (1940) has reviewed the physiological evidence regarding the caudal outflow of the hypothalamus. He concludes that this runs through 'both the central and the tegmental portions of the midbrain' and that these connexions concentrate again in the tegmental regions of the pons. Although it is clear that the caudal outflow of the hypothalamus divides into a periaqueductal and a tegmental portion the relationship between these two parts is not at all clear at present. It seems likely that a number of the fibres that enter the midbrain tegmentum pass through this into the periaqueductal grey, and on the other hand, the caudal connexions of the periaqueductal grey appear to pass through the tegmentum of the pons.

Some of the fibres that run caudally from the premamillary hypothalamus pass directly into the mamillary region (see also Cajal, 1911; Bodian, 1940). A number of these fibres end in the dorsal premamillary nucleus, the pars medianus and the anterior part of the pars medialis of the medial mamillary nucleus. An ending in the lateral mamillary nucleus, the pars posterior or the pars lateralis of the medial nucleus cannot be completely excluded at present.

These hypothalamo-mamillary fibres show that the mamillary bodies are, at least in part, under the direct influence of the premamillary hypothalamus. A consideration of other hypothalamic connexions shows an even closer relationship between the premamillary and the mamillary parts of the hypothalamus. The dorsal tegmental nucleus lies at the caudal end of the periaqueductal grey and must be closely related to the efferent, periventricular outflow of the hypothalamus. The nucleus receives some of the fibres of the mamillo-tegmental tract and it gives origin to some of the fibres of the mamillary peduncle (Guillery, 1956, see also *rat* 331 of the present series). The mamillary peduncle ends in the anterior part of the medial mamillary nucleus and in the lateral mamillary nucleus; the mamillo-tegmental tract probably arises from the same parts of the mamillary region and the hypothalamo-mamillary fibres also end in the anterior part of the medial mamillary

nucleus. This part of the mamillary region must thus be closely related to the activity of the hypothalamus and its caudal periventricular outflow. It receives afferent fibres from the hypothalamus and from cells lying on the caudal hypothalamic outflow and it also sends efferent fibres to the latter.

These interconnexions suggest that the mamillary bodies play a part in controlling the caudal outflow of the hypothalamus but at present there is no direct evidence to support such a view. However, it is of interest to note that the part of the mamillary region most intimately concerned with these hypothalamic connexions also sends impulses via the anteromedial thalamic nucleus to the anterior cingulate cortex (Powell & Cowan, 1954; Rose & Woolsey, 1948), and that it is possible to obtain visceral responses from stimulation of this cortical area (Kaada, 1951).

The ascending fibres in the medial forebrain bundle

The medial forebrain bundle has been studied in considerable detail by a large number of workers (for references see Ingram, 1940), but the great majority of these studies have been based on normal material in which it is extremely difficult to separate individual fibre groups or to be certain of the direction of conduction. Most observers have described a number of descending components in the medial forebrain bundle and only a few have mentioned possible ascending fibres.

Cajal (1955) described a coarse-fibred, ascending or sensory pathway, which forms a large part of the radiation of Zuckerkandl in the medial parts of the septum. The septal terminals of these fibres form a loose plexus in the region between the genu of the corpus callosum and the fornix. Cajal also described a second ascending pathway, finer than the first and ending in the lateral parts of the septum. He considered that both pathways arise in the region of the cerebral peduncle. There can be little doubt that these two pathways correspond to the bundles that have here been called the medial and the lateral components of the medial forebrain bundle. It is most probable that the cells of origin of the medial component lie in the midbrain or pons, while those of the lateral component lie in the premamillary hypothalamus.

Of the workers who have studied ascending Marchi degeneration in the medial forebrain bundle after hypothalamic lesions Beattie *et al.* (1930) mention ascending granules passing to the supraoptic commissures, Morin (1950) does not describe ascending degeneration passing beyond the preoptic region, and Nauta (1946) describes some fibres which reach the septum. It is probable that the fine lateral component would not show in a Marchi preparation but it is difficult to account for the absence of the coarse medial component in such preparations.

Most of the coarse ascending degeneration in the medial component of the medial forebrain bundle ends in the medial septal nucleus, but a few fibres also continue into the hippocampus. The medial septal nucleus sends its axons into the hippocampal formation (Daitz & Powell, 1954) and thus lies on an ascending pathway that passes from the pons or midbrain to the hippocampus. Most of the fibres that form this pathway relay in the medial septal nucleus but some may relay in the lateral hypothalamus and the nucleus of the diagonal band and a few may reach the hippocampus without relay.

Green & Arduini (1954) have shown that hippocampal activity can be influenced by an ascending system that passes through the midbrain tegmentum, hypothalamus and septum. There can be little doubt that the medial component of the medial forebrain bundle plays an important part in this ascending system.

The lateral septal nucleus receives afferent fibres from the hypothalamus via the lateral component of the medial forebrain bundle. It also receives afferents from the fimbria (Daitz & Powell, 1954; Sprague & Meyer, 1950) and may send efferent fibres to the hypothalamus but this last connexion needs further investigation.

Both the medial and the lateral septal nuclei thus lie on pathways that link the hippocampus and the hypothalamus, and these are further linked by the interconnexions that pass through the mamillary bodies. Further studies of the septal connexions appear to be particularly relevant to an understanding of the hippocampo: hypothalamic relationships.

The present results show that the fibres which pass through the posterior hypothalamus include the caudal outflow of the hypothalamus, the mamillary system of connexions and an ascending group of mesencephalo-septal fibres. A second ascending group of hypothalamo-septal fibres arises at more rostral levels. The hypothalamo-thalamic fibres have not been included in the present study, but they must be regarded as a further important hypothalamic connexion. The distribution of these various fibre groups may account for the major regional differences that can be found in stimulation and ablation experiments (e.g. Ranson & Magoun, 1939; Hess, 1954; Nauta, 1946), but it is probable that another method of investigation will be necessary to account for the finer differences.

SUMMARY

1. Degeneration in the hypothalamic connexions of the rat has been studied by the method of Nauta & Gygax (1954).

2. Degeneration in the mamillo-thalamic tract has been traced to each of the three homolateral anterior thalamic nuclei and to the contralateral anterodorsal nucleus.

3. Degeneration in the mamillo-tegmental tract has been traced to the dorsal tegmental nucleus and to the tegmental reticular nucleus. There is no evidence for a heavy contribution to the deep tegmental nucleus. No mamillo-tegmental fibres were traced to more caudal levels.

4. Ascending fibres in the medial forebrain bundle can be divided into two distinct groups. A fine fibred hypothalamo-septal group ends in the lateral septal nucleus and a coarse fibred mesencephalo-septal group ends in the medial septal nucleus.

5. Two groups of descending fibres pass from the hypothalamus to the midbrain. One passes into the periaqueductal grey and the other passes into the ventromedial parts of the midbrain tegmentum. Some of the latter, however, run through the tegmentum and join the caudal end of the periaqueductal grey. It has not been possible to trace any hypothalamic fibres beyond the level of the seventh nerve.

6. A number of fibres pass from the premamillary hypothalamus to the anterior part of the medial mamillary nucleus.

7. The present results show that there is a complex system of interconnexions linking the medial forebrain bundle, the periventricular system and the hippocampo: mamillary system of connexions.

I wish to thank Prof. J. Z. Young for his advice and encouragement, Miss B. Shirra for technical assistance, Miss E. R. Turlington for the drawings and Miss T. Marchment for the photomicrographs.

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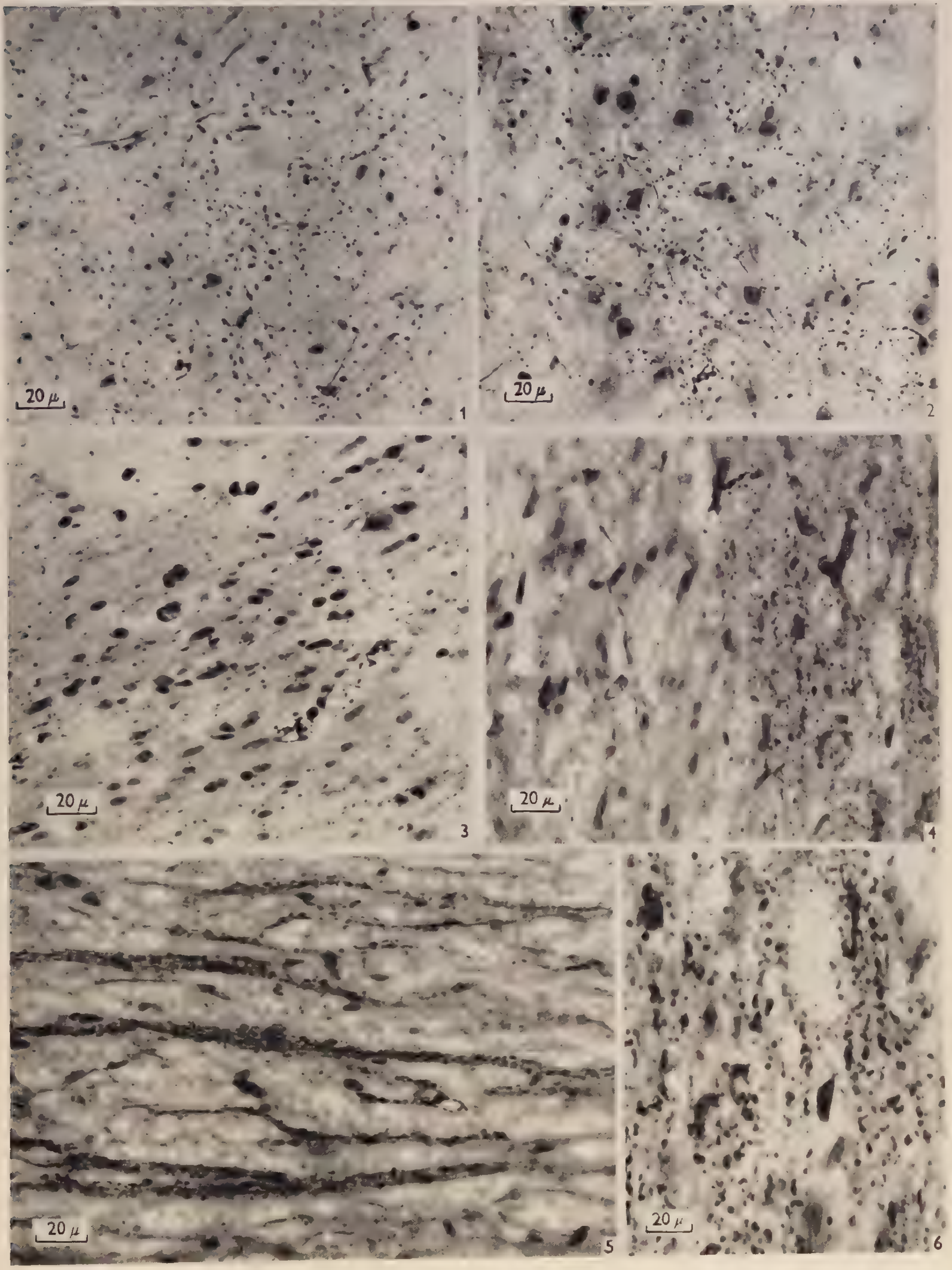
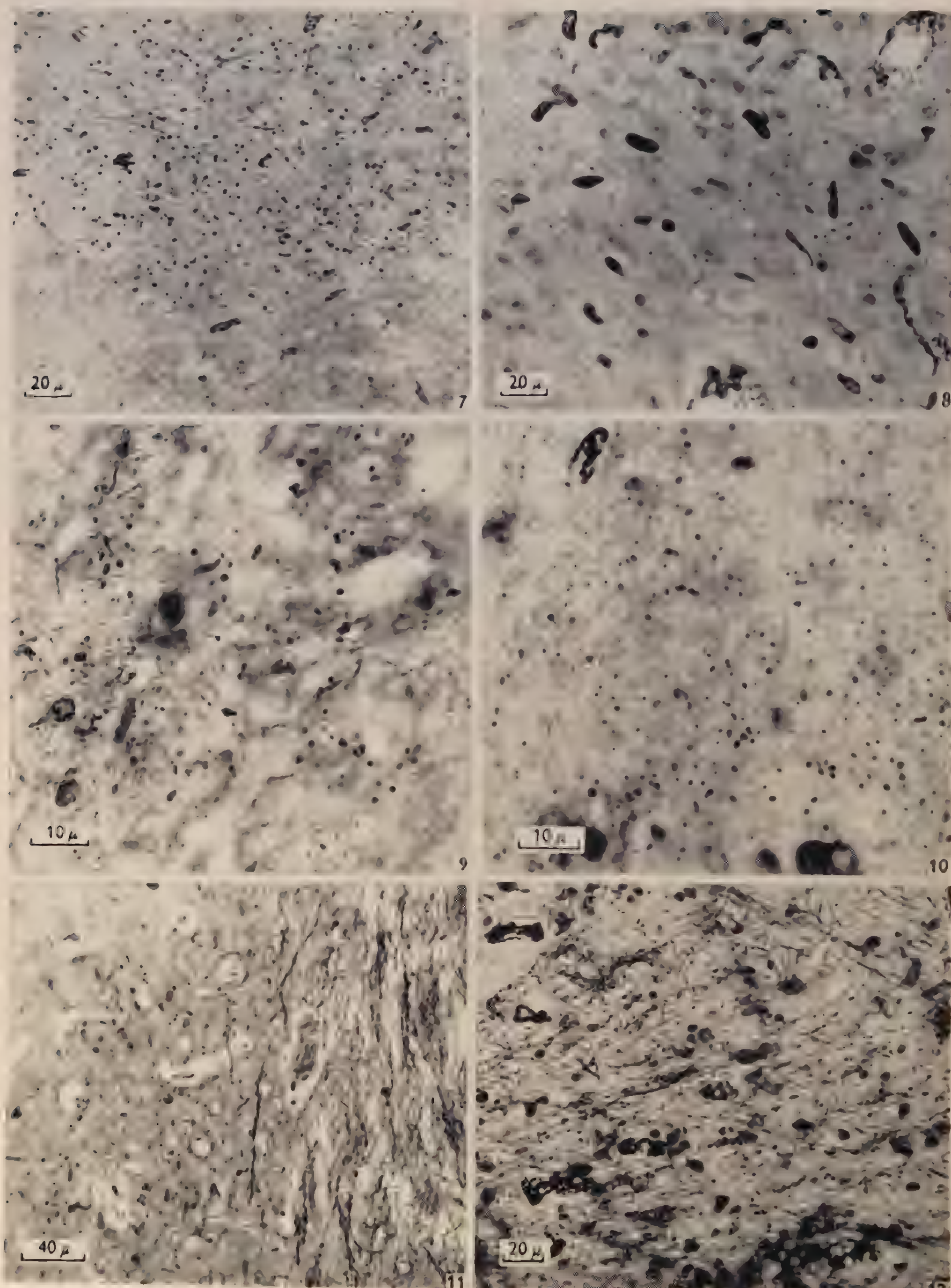
LIST OF ABBREVIATIONS

a.c.	Anterior commissure
a.h.	anterior hypothalamus
arc.	arcuate nucleus of the hypothalamus
b.a.c.	bed nucleus of the anterior commissure
c.c.	corpus callosum
c.g.	central (periaqueductal) grey matter
c.i.	internal capsule
c.n.	caudate nucleus
dl.t.n.	dorsal tegmental nucleus
dm.	dorsomedial hypothalamic nucleus
dp.t.n.	deep tegmental nucleus
d.s.c.p.	decussation of the superior cerebellar peduncle
f.	fornix
fi.	fimbria
h.	habenular nuclei
h.c.	habenular commissure
h.p.	habenulo-peduncular tract
i.c.	inferior colliculus
ip.n.	interpeduncular nucleus
l.m.n.	lateral mamillary nucleus
l.s.	lateral septal nucleus
m.b.	mamillary bodies
m.l.	medial lemniscus
m.l.b.	medial longitudinal bundle
m.p.	mamillary peduncle
m.s.	medial septal nucleus
m.t.	mamillo-thalamic tract
n.ac.	nucleus accumbens
n.d.b.	nucleus of the diagonal band
o.	nucleus ovoideus
o.t.	olfactory tubercle
p.c.	posterior commissure
p.h.	posterior hypothalamus
p.l.	pars lateralis of the medial mamillary nucleus
p.m.	pars medialis of the medial mamillary nucleus
pm.d.	dorsal premamillary nucleus
p.mn.	pars medianus of the medial mamillary nucleus
p.n.	pontine nuclei
p.p.	pars posterior of the medial mamillary nucleus
pm.v.	ventral premamillary nucleus
p.v.	hypothalamic paraventricular nucleus
s.c.	superior colliculus
s.o.n.	supraoptic nucleus
t.d.	tegmental decussations
t.r.n.	tegmental reticular nucleus
vm.	ventromedial hypothalamic nucleus
z.i.	zona incerta
III	nucleus of the third cranial nerve
IV	nucleus of the fourth cranial nerve



GUILLERY—DEGENERATION IN HYPOTHALAMIC CONNEXIONS OF ALBINO RAT

(*Facing p.* 114)



GUILLERY—Degeneration in hypothalamic connexions of albino rat

EXPLANATION OF PLATES

Plate 1

Fig. 1. Degeneration in the homolateral anterodorsal thalamic nucleus of rat 280.

Fig. 2. Degeneration in the contralateral anterodorsal thalamic nucleus of rat 280.

Fig. 3. Degeneration in the periaqueductal grey of rat 350.

Fig. 4. Degeneration in the tegmental reticular nucleus of rat 350. Note the sharp boundary to the zone of degeneration.

Fig. 5. Degeneration in the mamillo-tegmental tract rostral to the decussation of the brachium cunjunctivum in rat 350. Notice the scattered bundles that are formed by this tract.

Fig. 6. Degeneration in the tegmental reticular nucleus of rat 350. The same field as fig. 4 at a higher magnification.

Plate 2

Fig. 7. Moderate to dense degeneration in the dorsal tegmental nucleus of rat 302.

Fig. 8. Sparse degeneration in the dorsal tegmental nucleus of rat 350.

Fig. 9. Degeneration in the medial septal nucleus of rat 286.

Fig. 10. Degeneration in the lateral septal nucleus of rat 286.

Fig. 11. Degeneration in the medial septal nucleus close to the fornix in rat 280. Note the normal fornix fibres on the right.

Fig. 12. Degeneration radiating dorso laterally through the supramamillary region in rat 286.

(Note: All the sections have been stained by the method of Nauta and Gygax.)

THE MALE REPRODUCTIVE TRACT OF THE FOWL

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The male reproductive organs of the fowl have been studied by Kaupp (1915), Gray (1937), Burrows & Quinn (1937), Parker, McKenzie & Kempster (1942), and Bradley (1950), mainly by histological methods of fixation and staining; little attention has been paid to details from the point of view of the origin of semen constituents. Hence, in the course of investigations on the development of artificial insemination methods in the fowl, it was thought appropriate to examine the male tract and associated organs, using gross dissection, cytochemical and histological methods in an attempt to locate sites of secretory activity, as a necessary preliminary to studies of ejaculation phenomena and of chemical analyses of fowl semen in relation to the *in vitro* activity of spermatozoa. Fowl spermatozoa have been shown to be particularly difficult to store *in vitro*. Apart from their inherent structural peculiarities, this difficulty might be due to constituents of the accessory fluids collected by the massage method; collectively, these have been taken to represent the seminal plasma. It is therefore desirable to locate in the male tract and associated organs all possible sources of secretions which could gain access to semen collecting vessels, so that a subsequent assessment can be made of the efficiency of semen collection by massage (Burrows & Quinn, 1935, 1937).

The organs in the male cloaca are interesting and need special attention when discussing massage collection. Nishiyama (1950*a*) has described variations of phallus form in the fowl; during sexual excitation the phallus swells by engorgement with lymph from the internal pudendal artery, and this process, together with relaxation of the posterior retractor penis muscle and the engorgement of the lymph folds in the cloaca (Nishiyama, 1950*b*, *c*) contribute to the protrusion of the phallus. Lymph folds and vascular bodies are linked with lymph channels of the phallus and are considered by Nishiyama (1954) as analogues of accessory reproductive organs of mammals, since they showed a positive growth response to male hormone administration. It is generally held that there is an analogy between the enlarged, distal portions of the vasa deferentia of the bird and the seminal vesicles of the mammal (Riddle, 1927; Bailey, 1953). On the other hand, as Grecka (1933) and Munro (1938) have stated that there appear to be no glands corresponding to those of the mammalian seminal vesicles in the fowl there is confusion as to the existence of homologous or analogous accessory glands in the two classes of vertebrates. During sexual excitation the lymph folds and vascular bodies are erected, together with the ejaculatory ducts. Nishiyama (1951, 1952*a*, 1955) observed a lymph-like fluid that flowed through their surface epithelium during massage collection of semen. Together with a little fluid secreted by the epithelial cells, this lymph constituted the so-called 'transparent fluid' of fowl semen and was considered by Nishiyama as true seminal plasma. Lake (1956) did not wholly agree with this

interpretation and so, in view of the importance of assessing the true nature of fowl seminal fluid, it was decided to investigate further the lymph folds and vascular bodies.

MATERIAL AND METHODS

Birds

All birds used were from the Brown Leghorn flock maintained at the Poultry Research Centre, Edinburgh.

(1) *Gross dissections.* Seven-month-old cockerels were used for observations on the anatomy of the male organs at the height of the reproductive season in March and April. The arrangement of seminiferous tubules in the testis was examined by the method used by Bailey (1953) to study testes of Fringillid birds. Immediately after killing, a solution of 2 g. Nigrosin in 20 ml. 0·85 % sodium chloride was injected into the tubules through a large bore needle inserted into the epididymal region. The testes were then taken out and kept in 40 % nitric acid at −2° C. for 24 hr., the tunica albuginea being punctured at several places to facilitate entry of acid. This treatment hardened and stained the tubules, and enabled the gross internal structure of the testis to be teased out and examined under a binocular dissection microscope.

(2) *Histochemical analysis of various parts of the urinogenital tract.* One-year-old males were used for histochemical examination of the epithelia lining the reproductive and urinary tracts. Immediately after killing, by dislocation of the neck vertebrae, pieces of testis, epididymal region, upper and lower vas deferens, ureters, phallus, lymph folds and vascular bodies were fixed in appropriate solutions for 24 hr. Frozen sections of each region of the tract were also prepared after formol-calcium fixation (Baker, 1946). After fixation in Carnoy-Lebrun fluid (McClung, 1950) saturated with mercuric chloride, Masson's trichrome and Ehrlich's haematoxylin and eosin staining methods were used to examine the distribution of muscle, connective tissue and fibrous tissue. In addition, the following staining procedures were used.

Acid and alkaline phosphatase. In the mammalian reproductive tract, phosphatases are secreted mainly by cells in specific accessory glands. Acid phosphatases are secreted by the prostate gland, and alkaline phosphatases by the seminal vesicles (Mann, 1954). The presence or absence of phosphatases in the lymph folds and vascular bodies of the fowl cloaca would give some indication as to whether or not these organs are analogous to the seminal vesicles, prostate glands or other accessory glands of mammals. For alkaline phosphatases the method of Gomori (Pearse, 1953) on paraffin embedded sections was used; fresh tissue pieces, not exceeding 2 or 3 mm.^{3}, were fixed in acetone at 2° C. On similar sections Gomori's method (Pearse, 1953) for acid phosphatases was employed. For both enzymes the sections were incubated in the substrate media for 24 hr. to get maximal reaction (Rollinson, 1954). Control sections were incubated for the same length of time in the buffer medium without substrate.

Lipids. By the term 'lipids' is meant all material (*a*) reacting positively with Sudan black B in frozen sections and extractable by acetone, ether, xylene, benzene or pyridine; and (*b*) all 'bound lipid' material not easily soluble in orgainic solvents

after formalin fixation and reacting in a manner characteristic of glycolipids and phospholipids.

Frozen sections of tissue after formol-calcium fixation were used for (1) Schultz test for unsaturated steroids, principally cholesterol (Glick, 1949), and (2) examination with the polarizing microscope. It has been shown by Cain (1950) that many lipids become crystalline in fixed preparations, and spherocrystals showing the black cross of polarization are formed by steroid esters and phosphatides. To look for the presence of such compounds, frozen sections of tissue fixed in formol-calcium were mounted in glycerine jelly and examined with polarized light.

Previous experience had shown that, in certain regions of the male reproductive tract, some lipid existed which was not easily soluble in fat solvents; pieces of each organ were therefore fixed in formol-calcium for 24 hr., washed in chilled acetone for two periods of 1 hr. each, placed in two or three changes of tertiary-butyl alcohol and then embedded in paraffin wax. Sections were subsequently stained with Sudan black B; it was considered that a positive reaction demonstrated the presence of a glycolipid, or a similar complex lipid. Pearse (1953) has shown that phosphatides and cerebrosides (glycolopids) give positive reactions to Sudan black in formol-fixed paraffin sections. Baker's acid haematin and pyridine extraction test (Baker, 1946; Cain, 1947) was used to examine the presence of phospholipid and glycolipid as both are extracted with pyridine treatment, but only the former gives a positive reaction with acid haematin.

Staining for general lipid material was done on fixed, frozen sections using Nile blue and Sudan black B (Pearse, 1953).

Muco-substances. It is generally recognized that mucopolysaccharides (Leblond, 1950), muco-proteins and lipo-protein-polysaccharides form the basis of some mucoid secretions. The function of these substances is little understood. Mucopolysaccharides are present in the pre-sperm fraction of bull ejaculates (Lutwak-Mann & Rowson, 1953). To identify the sites of production of these types of secretion in the male fowl tract, pending a more detailed biochemical analysis, the following histological tests were applied: (*a*) Lison's method (Glick, 1949) using toluidine blue metachromatic staining after fixation in equal volumes of basic lead acetate and 15 % neutral formalin. This method is considered to reveal mucopolysaccharide-sulphate compounds and possibly other acid mucopolysaccharides (Wislocki & Singer, 1950; Pearse, 1953); (*b*) after fixation in Orth's fluid, periodic acid treatment followed by fuchsin-sulphurous acid (FSA) (Hotchkiss, 1948; McManus, 1946), modified by Leblond & Clermont (1952), was used for detecting suitably reacting mucopolysaccharide which was not principally basophilic or metachromatic. Saliva, as a source of α-amylase, was used on control sections to determine how far glycogen was the basis of FSA-reactive material. It is known that certain monosaccharides, polysaccharides, mucoproteins, phosphorylated sugars, cerebrosides and inositol-containing lipids (Gersh, 1949) can give a positive FSA-reaction, and so with due consideration of the extent of solubility of these compounds in the fixation and staining fluids used, the colour reaction also served as a general indication of the presence of these types of compounds in the genital tract.

RESULTS

Gross morphological features of the male reproductive tract in the cock

Pl. 1, fig. 1, is a drawing from a dissection of a 7-month-old Brown Leghorn male weighing 2200 g. at the height of the reproductive season in Edinburgh in March. The paired testes (4·5 cm. long and 2·5 cm. broad) are internal and attached by connective tissue to the dorsal body-wall slightly medial to the anterior ends of the kidneys. The left testis (14 g.) was slightly smaller than the right (14·75 g.). The anterior portions were adjacent to the abdominal air sacs. Internally they resembled those of the Fringillid birds (Bailey, 1953) and differed from the mammalian testes. The membranous tunica albuginea covering the mass of seminiferous tubules is extremely thin. The tubules form an anastomosing network, are not discrete as in the mammal and are impossible to tease out individually (Pl. 1, fig. 2). Under the binocular dissecting microscope, portions of the tubules appeared swollen, possibly indicating sites of active multiplication of germ cells. They discharge their contents into the cavernous spaces of the rete testis and then into the extra-testicular vasa efferentia. The latter, with some exceptions (Stoll & Maraud, 1955), connect the seminiferous tubules with the ductus epididymis. Capillaries, arterioles and venules of the testicular artery and vein ramify throughout the intertubular tissue of the testis, and also in the tunica albuginea. Unlike that of the mammal, the active fowl testis is not firm to the touch, and when cut with a scalpel extrudes an abundance of milky fluid composed of lipoprotein material and spermatozoa from the seminiferous tubules. In contrast to the mammals, the ductus epididymis is extremely short and is not divided into caput, corpus or tail regions (Pl. 1, fig. 2).

The vas deferens receives the contents of the epididymis and is the main storage organ for spermatozoa in the cock. In the dissected bird it was a long, coiled tube running for about 9·5 cm. along the ventral aspect of the kidney. From midway along its length it ran parallel with the main ureter to the cloacal region (Pl. 1, fig. 1). The distal end was greatly thickened owing to an increase in musculature and connective tissue which will be described later. Contrary to the mammals, there was no region to compare anatomically with the ampulla. Each vas deferens connected directly with an erectile ejaculatory duct which protruded into the urodaeum of the cloacal chamber. The ducts were conical in shape, and those illustrated in Pl. 1, fig. 1, were 2·5 mm. long in the flaccid state.

The ureteral openings to the urodaeum were in close proximity, and medial to, the base of the ejaculatory ducts. The rectal opening into the coprodaeum lay posteriorly in the midline of the cloacal chamber. The phallus of the cock is composed of a central 'white body' and lateral 'round folds', and is situated in the anterio-ventral part of the cloacal chamber (Pl. 1, fig. 1). Discrete lymph folds lie posteriorly to the round folds and in front of the ejaculatory ducts. Vascular bodies forming a ring of erectile tissue lay behind the ejaculatory ducts and encircled the posterior aspect of the urodaeum. Nishiyama (1955) described vascular bodies as being two discrete bodies surrounding the base of each ejaculatory duct only.

Histological and histochemical characteristics of testis, epididymis, vas deferens and certain other parts of the reproductive tract

Testis. As already mentioned, the fowl testis is softer than that of mammals, and for this softness several reasons may be suggested. First, contrary to the condition in mammals, the tunica albuginea is extremely thin and does not appear to give off any connective tissue septa dividing the tubules into lobules (Pl. 1, fig. 3). In the inactive testis, where intertubular connective tissue is more prominent, there is also no sign of these septa and so, it is concluded, their absence in active testes is not just apparent owing to great distension of the tubules. Secondly, the seminiferous tubules of the fowl testis are proportionately larger in diameter than those of some mammals. Thirdly, one gets the impression by comparing the fowl seminiferous tubule (Pl. 2, fig. 4) with that of a mammal having a comparable testis size, that the spermatocytes of the bird are smaller, and a more rapid process of germ cell division is evident from the number of cells seen per unit area within the tubules.

In the tubules of the active testis there was no material showing birefringence under the polarizing microscope, or which stained positively with acid haematin, thus indicating the absence of cholesterol and phospholipids. Similar observations with regard to cholesterol were made by Marshall & Coombs (1952) on wild bird testes, and Mancini, Nolazco & de la Balze (1952) on human testes. Traces of such material, were, however, detected in the intertubular tissue. The Sertoli cells appeared to contribute some type of lipid to the contents leaving the seminiferous tubules; their cytoplasm was very extensive and appeared to ramify between all the cells of the germinal epithelium. With phase-contrast microscopy large lipid droplets were seen in preparations from seminiferous tubules of fresh testis material. After fixation of the testis in formol-calcium, frozen sections stained with Sudan black B showed lipid chiefly at the sites surrounding the transforming spermatozoa in the lumen and interspersed between the later spermatocytes. Tissue fixed in formol-calcium, and treated with chilled acetone prior to paraffin embedding, still showed in these places a positive, but slightly reduced, reaction to Sudan black. In the same areas there was also a positive FSA reaction indicating the presence of mucopolysaccharide or aldehyde groups produced from complex lipids (Pearse, 1953). It was difficult to decide whether the FSA-positive material was in the cytoplasm being shed by the spermatozoa in the latter stages of their formation or whether it was in the Sertoli cell cytoplasm.

After salivary digestion the FSA-picture in the testis was altered only very slightly from that given by the control sections. If this indicates solely loss of glycogen, then the amount of this substance present is small. Metachromasia, indicating acid mucopolysaccharide, was confined to the basement membrane of seminiferous tubules, and to chromosomes in the spermatocytes during the metaphase stage of division. The results of staining with Nile blue showed that there was a slight increase of acidic lipid material in cells towards the lumen of tubules.

Alkaline phosphatase in the testis was present in the cytoplasm and in the nuclei of cells of the intertubular tissue. A much weaker reaction of the enzyme was observed in the nuclei of germ cells which formed the basal layer in tubules. Very feeble reactions were given by the cytoplasm and nuclei of Sertoli cells, secondary

spermatocytes and spermatids. The acid phosphatase reaction was feeble in the basal cell nuclei. These findings would indicate that the fowl testis is similar to most mammalian testes with regard to the distribution of the alkaline and acid phosphatases (Rollinson, 1954; Wolf, Kabat & Newman, 1943; Wislocki, 1949).

From the seminiferous tubules to the rete testis the transitional stage in the change of the epithelium was from the typical stratified germinal type to the single layer, cuboidal cell type of the rete. At the transitional stage modified Sertoli cells predominated and their cytoplasm was marked by more positive reactions to FSA and Sudan black staining. Large secretory droplets from these cells, which stained positively with Sudan black after acetone treatment and embedding in paraffin wax, were often seen in the lumen. This is considered to indicate the presence of a compound lipid similar in nature to that produced in the seminiferous tubules. The lipid in the rete epithelium appeared to be mostly part of the cell structure and not secretions.

Epididymal region. Since there is no long, highly tortuous ductus epididymis in the cock, the term 'epididymal region' is used in the present work to describe the small group of tubules lying on the hilum of the testis. The structure is comprised of many short vasa efferentia joining the ductus epididymis (Stoll & Maraud, 1955). The whole is embedded in connective tissue and firmly anchored to the dorsal body-wall. On the left side most of the adrenal gland is embedded in the connective tissue of the proximal epididymal region.

The several types of vasa efferentia had a lining epithelium exhibiting in places intense holocrine secretion. Intra-epithelial glands occurred, and long tufts of stereocilia were carried on the free surface of some of the cells (Pl. 2, fig. 5). Abundant lipid-protein material was present (Pl. 2, fig. 6), and some of it, after fixation in formol-calcium and subsequent treatment with acetone before embedding in paraffin wax, was still revealed with Sudan black. FSA-positive material was present in the lipid droplets which indicates the presence of either aldehydes produced from a complex lipid or a mucopolysaccharide fraction (Pl. 2, fig. 7). There was no demonstrable phospholipid revealed by the acid-haematin test. A few smooth muscle cells appeared beneath the basement membranes of the vasa efferentia.

There were parts of tubules in close proximity to the basement membrane of the epithelium of some of the vasa efferentia, and large lipid droplets also occurred in their cytoplasm (Pl. 2, fig. 6). It is possible that these represent the satellite tubules of the vasa efferentia. During the active season the epithelial cells of the vasa efferentia were to be seen in various representative states of building up and breaking down. This points to marked functional activity, during the course of which it seems likely that much lipid-protein-polysaccharide, together with other cytoplasmic inclusions, is secreted.

Occasionally groups of cells appeared in the connective tissue of the epididymal region which were positive to the Schultz test and also birefringent, thus indicating the presence of cholesterol esters. These may represent either 'le paradidyme' or the 'vasa aberrantia of rete' (Stoll & Maraud, 1955). In the course of dissection isolated small yellow patches were noticed in the connective tissue of the epididymal region. Some of them were undoubtedly nodules of adrenal tissue, but some most likely represent rudimentary tubules from the mesonephros which produce the buff-

coloured droplets that appear in the semen, especially of young cockerels just beginning to be sexually active.

The ductus epididymis generally had a smooth, circular outline and had a lining epithelium of pseudo-stratified columnar cells (Pl. 2, fig. 8). There was no indication of secretion from the cells. There did not seem to be sufficient specific evidence of glycogen secretion in the ductus epididymis or vasa efferentia tubules comparable with what is thought to occur in the dog and rabbit (Nicander, 1954), and in man (Montagna, 1952). FSA-stained sections after saliva treatment did not produce any picture different from that of the untreated sections. In the ductus epididymis the lipid and FSA-positive material was greatly reduced in the epithelium, and appeared to be confined to the cell framework only.

A weak acid phosphatase reaction was observed in the secretions of cells in some of the vasa efferentia; in the ductus epididymis the reaction was negative. This is contrary to the condition found in man (Montagna, 1952), rabbit, guinea-pig and mouse (Wolf *et al.* 1943). Alkaline phosphatase appeared to be faintly scattered throughout the cytoplasm of all cells in the epididymal region.

Vas deferens. From the evidence already given it is likely that spermatozoa are bathed in a lipo-mucoprotein medium when entering the vas from the ductus epididymis. At the junction of the vas deferens with the ductus epididymis the epithelium was slightly folded, and large vacuoles appeared to be formed at the free surface of the cells and to be extruded into the lumen. The upper region, prior to the distended part, had a relatively wide lumen (Pl. 2, fig. 9) and an epithelium showing a little lipid material scattered throughout the framework of the cells only. The short distal region was characterized by an increased thickness of the fibrous tissue of the submucosa and the outer smooth muscle (Pl. 3, fig. 10). There was a scanty distribution of lipid in cells of the vas but it did not seem to form an extensive secretion to the seminal fluid. Only very weak or absent reactions for acid and alkaline phosphatases were demonstrated in the epithelium of most of the vas, but occasionally isolated areas in the distal region were found secreting some acid phosphatase. After performing the phosphatase tests a variable amount of yellow-brown coloration was seen in the tissue due to non-specific adsorption of sulphide precipitate on muscle, collagen, etc. The areas where the enzyme was taken to be definitely present were coloured dark brown or black and such was the case in the above-mentioned areas of the distal vas after the test for acid phosphatase. The reaction in most of the epithelium of the vas was more towards yellow-brown coloration of nuclei and cytoplasm, thus indicating little or no acid or alkaline phosphatase to be present. A little FSA-positive material was present in cell cytoplasm, and some was seen amongst spermatozoa when the latter were retained in sections of the distal vas deferens. Blebs of cell extremities were occasionally to be seen extruding into the lumen of the vas deferens at this level (Pl. 3, fig. 11), and thus it is likely that any intracellular enzyme would be shed into the lumen along with cytoplasmic constituents and their breakdown products.

At the junction of the vas deferens with the ejaculatory duct there was a noticeably small storage space for spermatozoa but the vas as a whole formed the main reservoir. There was no mucopolysaccharide present in the epithelium of this region and only a little faintly scattered lipid was visible within the cells. At the distal end

of the vas deferens there was no glandular epithelium comparable with that of the ampulla of mammals.

Ejaculatory ducts. Each vas deferens joined the corresponding erectile ejaculatory duct which protruded into the urodaeum of the cloacal chamber (Pl. 1, fig. 1). The ducts were similar in structure to the penis of mammals in respect of subepithelial sinuses and tortuous arterioles and venules in the deep fibrous connective tissue of the submucosa (Pl. 3, fig. 12). The small blood vessels, and subepithelial sinuses, become engorged with blood during erection. The swelling of each ejaculatory duct appeared to be partly brought about by a contraction of the muscle of the vas deferens, forcing the spermatozoa into it. During collection of semen this can be clearly seen on occasions when lumbar stimulation causes filling of the duct and only gentle squeezing with the fingers is necessary to eject the semen into a container. The inner lining epithelium of the ejaculatory duct was of a pseudostratified columnar type; only a little scattered lipid and no mucopolysaccharide material was identifiable within the epithelial cells. The epithelium was much folded at the proximal end but was straightened out distally. The epithelium of the external surface of the duct contained mucin-secreting cells (Pl. 3, fig. 13). There was no acid or alkaline phosphatase reaction in the cytoplasm of the latter.

Lymph folds, vascular bodies and phallus. These tissue structures are considered together since they form what the author considers the most controversial elements in comparing the morphological and functional relationships of the reproductive tracts of birds and mammals. Together with the erectile, ejaculatory ducts they form the copulatory organ of the cock.

The vascular bodies have a surface epithelium of pseudo-stratified columnar cells. Glands are formed which sometimes extend into the submucosa, and many goblet cells are present (Pl. 4, fig. 14). The cells of the deep glands secrete an abundance of FSA-positive material that stains metachromatically (γ red) with Toluidine blue, indicating the presence of sulphated mucopolysaccharide (Pl. 4, figs. 15, 16). A little acidic lipid material is present also which stains with Nile blue. Neither phosphatase reaction is given by the cells. Many aggregations of lymphocytes occur in the submucosa, especially near to the ureteral openings (Pl. 4, fig. 14). Sinuses lined with endothelium and filled with blood are present underneath the basement membrane of the surface epithelium.

The epithelial cells of the ureters, which open into the urodaeum at the base of the ejaculatory ducts, also contain abundant sulphated mucopolysaccharide which forms part of the mucinous secretion. Aggregations of lymphocytes are present in the submucosa. The metachromatic reaction in the epithelium is equal in intensity to that of the vascular body epithelium. Only the nuclei of the lymphocytes give feeble phosphatase reactions.

The surface epithelium of the lymph folds is of the pseudo-stratified columnar type, and some of the cells secrete FSA-positive material which gives only a very slight metachromatic reaction (Pl. 4, fig. 17). This indicates that the mucin is less acidic in nature. There are no submucosal glands. Blood sinuses are present in the submucosa but are not so extensive as those in the vascular bodies. Very few smooth muscle cells are present beneath the basement membrane of the epithelium; it is suggested, therefore, that during normal copulation there is no forcible ejection of

secretion from the epithelial cells. This secretion could be passively extruded, as a result of the engorgement of the organ, and together with spermatozoa and fluids from the ejaculatory ducts, is most likely to be a component of the seminal fluid.

The phallus (Pl. 1, fig. 1) is bounded exteriorly by stratified squamous epithelium of the mucous membrane variety. Immediately beneath this, abundant muscle tissue, representing the posterior retractor penis muscle, is firmly anchored. Towards the interior of the cloaca the epithelium becomes similar to that of the lymph folds.

DISCUSSION

Although the present study has been confined chiefly to morphological features of the male fowl reproductive tract, it has revealed several facts which have a direct bearing on the problem of the physiological function of the various reproductive organs. The first point to be considered is the close relationship of the abdominal air sacs to the anterior poles of the testes. In wild birds, according to Cowles & Nordstrom (1946), the abdominal air sacs serve in a similar capacity as the scrotum does in the mammal. From anatomical investigations on the domestic fowl it is difficult to understand how the air sacs in their position could have much of a cooling action on the testes. Without rapid respiration induced by flying, the incoming air must be appreciably warmed by its passage through the lungs. Further physiological work is needed to investigate this aspect of avian reproduction.

Next to be considered is the presence of so-called seminal vesicles in the fowl. The common conception that the swollen distal portion of the vas deferens serves as a storage organ for spermatozoa in the fowl appears unlikely since the increased size is mainly accounted for by increased musculature and connective tissue in the subepithelial layers. The whole vas serves as a transitory storage place in the absence of an extensive ductus epididymis. This suggestion is in partial agreement with the findings of Nishiyama (1951) who found the greatest concentration of spermatozoa in the proximal end of the vas in the cock and not at its distal end. Morphologically there does not seem to be any evidence for drawing an analogy between the seminal vesicles of mammals and the swollen, distal parts of the vasa deferentia of the fowl, since the latter lack the glandular structure of seminal vesicles. Also, in the mammal, the latter are generally outgrowths and separate organs derived from the vas deferens; no such development occurs in the fowl. There is no secretion of alkaline phosphatases which in mammals generally are produced by the seminal vesicles (Mann, 1954). Bailey (1953), studying Fringillid birds, stated that the only reason he had for calling the distal vasa deferentia by the name seminal vesicles was because it was conventional to do so. In the expanding field of semen physiology this can lead to confusion when comparing the function and chemical composition of seminal fluids from various species of animals, and so, on present evidence and until more data are available as to the chemical nature of secretions produced in the fowl reproductive tract, it is considered preferable not to liken the distal vasa deferentia in the fowl to the seminal vesicles of mammals. The junction of the vas deferens with the ejaculatory duct might be considered as a contracted region similar to the ampulla of mammals. However, there are no glands extending into the submucosa, and the general epithelium shows no signs of secretory activity.

In the cock, it has been impossible to demonstrate any gland that compares morphologically with the prostate. In the mammal the latter is derived from urogenital sinus epithelium during embryogenesis, and in the mature animal the organ is composed of numerous tubulo-alveolar glands embedded in connective tissue through which smooth muscle septa run. The glands secrete material into the urethra through independent ducts. Smooth muscle contractions during copulation govern the process.

Nishiyama (1954) stated that the lymph folds and vascular bodies were analogous to accessory reproductive glands of mammals on the basis of their positive growth response to androgenic hormone. It is well known, however, that in mammals the penis and associated glandular structures, as well as the seminal vesicles and prostate gland, will respond to such hormone. It is, therefore, necessary to be more specific in definition. Unlike the seminal vesicles or prostate gland, the cloacal glands do not produce secretions of acid or alkaline phosphatases. The acid phosphatase appears to be a universal feature of the prostate gland (Wolf *el al.* 1943; Rollinson, 1954). By their position and also by the presence of much sulphated-mucopolysaccharide in the secretions of their glands, the vascular bodies seem more likely to be analogous with the mammalian bulbo-urethral glands. It is significant that Lutwak-Mann & Rowson (1953) found that in the secretion of such glands in the bull there is a fair quantity of combined hexosamine and glucuronic acid indicative of the presence of mucopolysaccharide. The pH of the fluid obtained from the bull was highly alkaline, pH 7·8, which corresponds with the figure pH 7·9 given by Nishiyama (1952*b*, 1954) for that produced by the lymph folds and vascular bodies of the fowl. Lutwak-Mann & Rowson suggested that as the secretions of the bulbo-urethral glands are ejaculated first they serve to clear and neutralize the contaminants of the urethra prior to the passage of the fraction of the ejaculate containing spermatozoa. In the fowl it is feasible that the secretions of the epithelial glands of the vascular bodies subserve a similar purpose of protecting the cloacal muscosa from damage by obnoxious constituents of the faeces and urine. In the submucosa of the lymph folds, but particularly in the vascular bodies, there are also numerous foci of lymphocytes.

The vascular bodies in the Brown Leghorn appear to form an almost continuous ring of tissue around the base of the ejaculatory ducts in the urodaeum. On morphological evidence it is suggested that their surface epithelial glands are related to the cloacal structures formed in embryogenesis and associated with the distal development of the urinary ducts. Their erectile properties, and those of the lymph folds, indicate a close analogy of these two structures with the penile, urethral portions of the mammal.

Although histochemical methods alone are not always adequate to demonstrate specific chemical compounds in tissue it is considered that they have, at least, helped in the present work to throw light on the general type of secretory material elaborated by the various parts of the male reproductive tract. Sufficient information has been obtained to serve as a general guide to the consideration of a future chemical analysis of semen constituents in relation to fowl sperm activity *in vitro*. The cytoplasm of Sertoli cells, of spermatids during the formation of spermatozoa, and cells of the vasa efferentia all appear to contribute material of a complex

lipo-protein-polysaccharide nature to the seminal fluid of the fowl; without, as yet, confirmatory results of chemical analysis, it would appear that a compound containing lipid and not very soluble in organic solvents is secreted. Inositol-containing lipids and cerebrosides are worthy of consideration. It is not generally stressed that testis products contribute to seminal plasma, however, Regaud (1902) was of the opinion that lipid of Sertoli cells actively contributed to the seminal secretion of the rat, and Lynch & Scott (1951) reported that, in the rat, lipid was stationary in Sertoli cells at times when spermatogenesis was inhibited, but in active seminiferous tubules lipid droplets were shed into the lumina. It is not considered that the observations of Coombs & Marshall (1956), indicating no lipid in active fowl testis tubules, are at variance with the findings of lipid in the present work. From the photomicrographs of their paper, some lipid is indicated, but it is insignificant in amount and of the wrong type for their hypothesis.

The epithelia of the proximal and distal vas deferens exhibit intense holocrine secretion. There is only a slight positive reaction with Sudan black and FSA, and so the organic secretion is largely composed of some other non-lipid compound possibly of a protein or polypeptide nature.

Compared to the mammalian reproductive tract there is no major gland secreting phosphatases into the seminal plasma of the fowl. The epithelia of parts of the vasa efferentia and parts of the vas deferens shed blebs of cytoplasmic material from the apices of cells which could contribute some intracellular phosphatase to seminal plasma. This does not necessarily mean that such material plays any direct, significant part in the metabolism of spermatozoa. In a cytochemical examination, one must be extremely cautious in ascribing any enzyme present in the seminiferous tubule specifically to either spermatozoa, or the cytoplasms of Sertoli or basal spermatogenic cells, since it is difficult to identify the cell types with the staining procedure used.

It is feasible that the complex lipo-protein in fowl semen, since it is not diluted with much accessory secretion, affords protection to the surface of spermatozoa and accounts partially for their relative resistance to temperature shock compared with most mammalian spermatozoa (Smith & Polge, 1950; Skaller, 1951; Lake, 1954). This is in accord with the view that some of the beneficial effect of adding egg yolk to mammalian semen diluents is due to the presence of complex lipids or lipoproteins which can afford protection to the spermatozoa (Mayer & Lasley, 1944; Blackshaw, 1954). The role of seminal fluid constituents in the maturation phenomenon of the spermatozoa in the epididymis and vas deferens, as well as a biochemical analysis of fowl semen and cloacal gland fluids, are at present under investigation.

SUMMARY

The structure of the reproductive tract of the cock has been examined to determine the nature of fluids which can gain access to collecting vessels during the massage method of semen collection. Although future chemical analyses are necessary to examine the precise nature of substances secreted into the seminal fluid, the organic matter appears to be mainly composed of complex lipid and protein-polysaccharide materials. It is derived from the seminiferous tubules and vasa efferentia. The

apices of cells in parts of the vas deferens break down and appear in this way to contribute material to the seminal fluid. It has been confirmed that in the cock there are no glands which are analogous to the seminal vesicles or prostate glands of mammals. Cytologically, the vascular bodies in the cloaca bear the closest resemblance to bulbo-urethral glands in respect of secreting abundant mucopolysaccharide. The lymph folds secrete mucin of a similar nature but, probably, less acidic in reaction.

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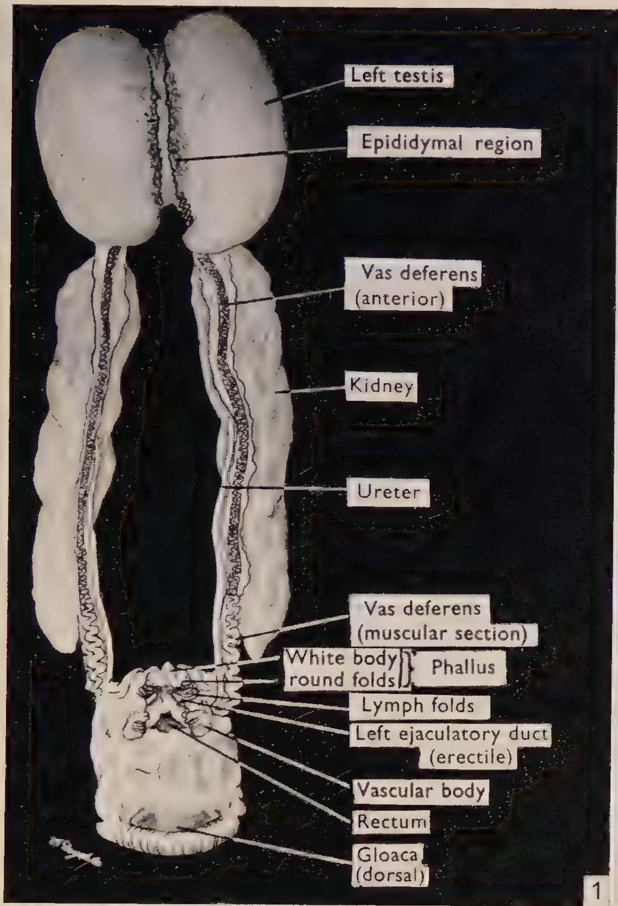

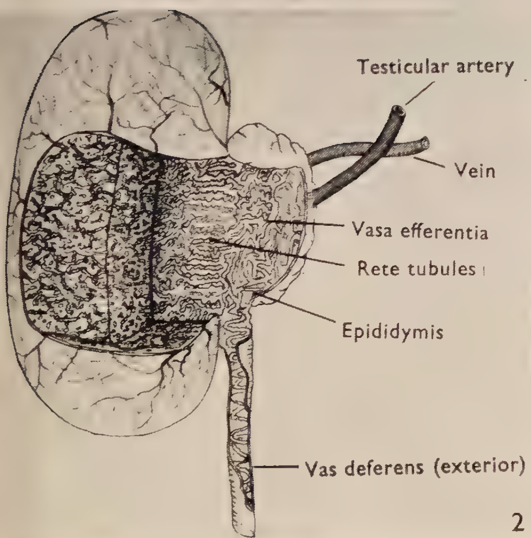

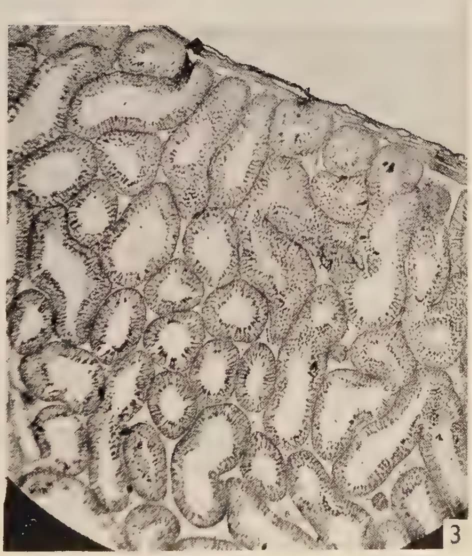

LAKE—The male reproductive tract of the fowl

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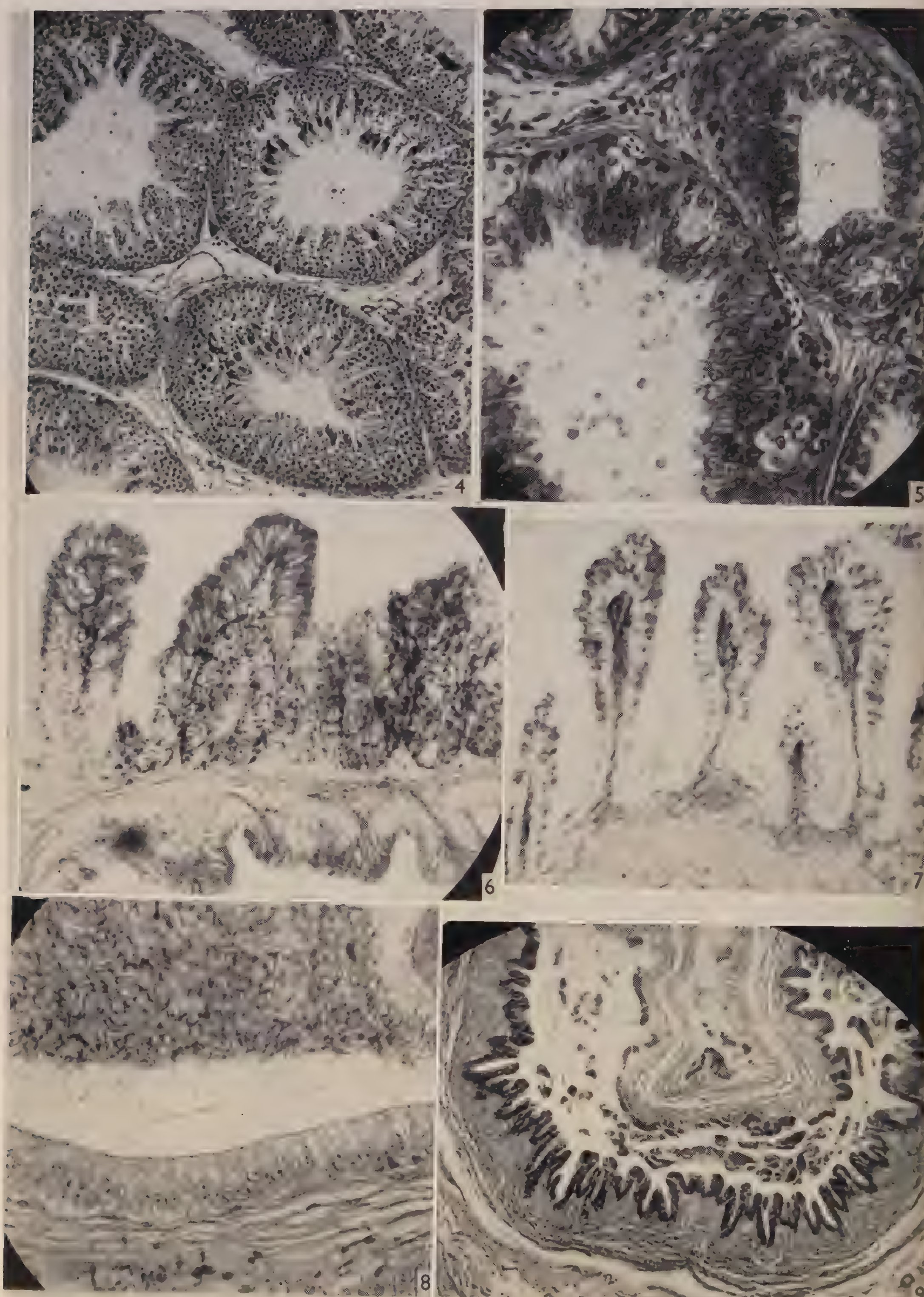

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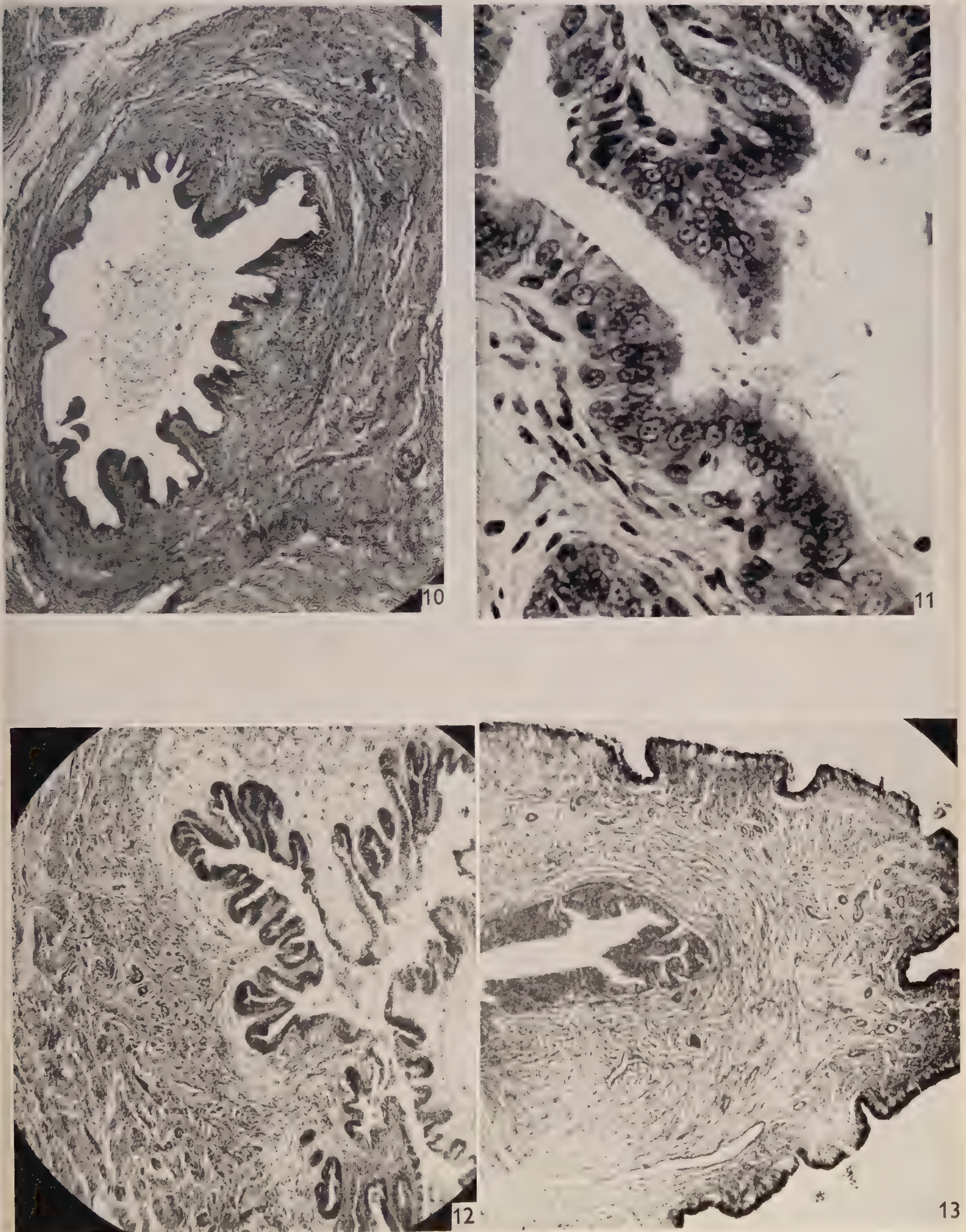
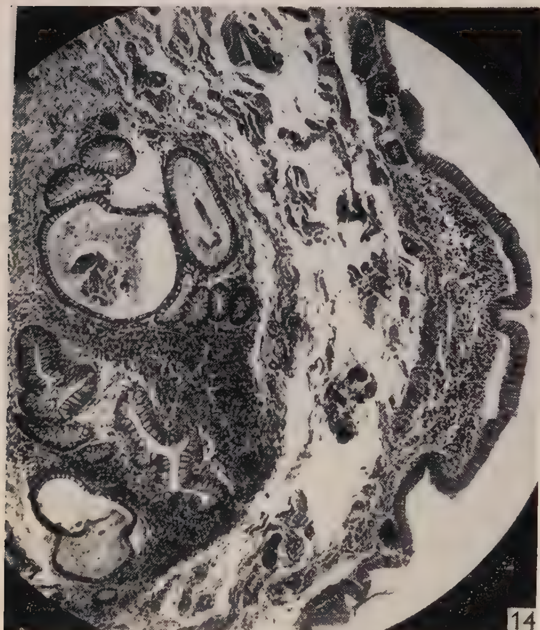
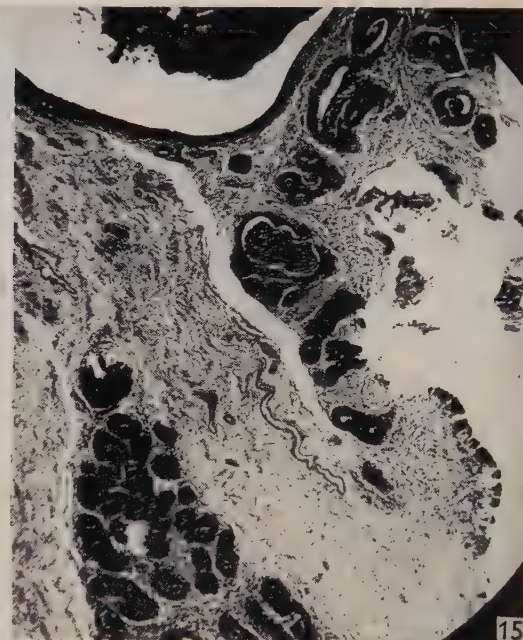
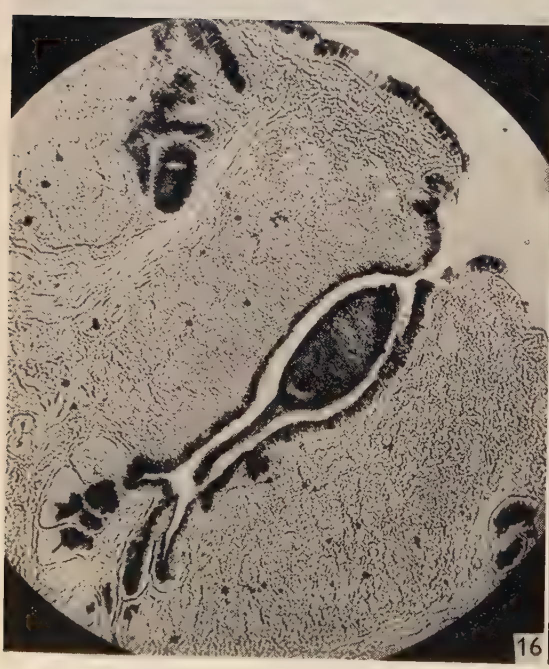
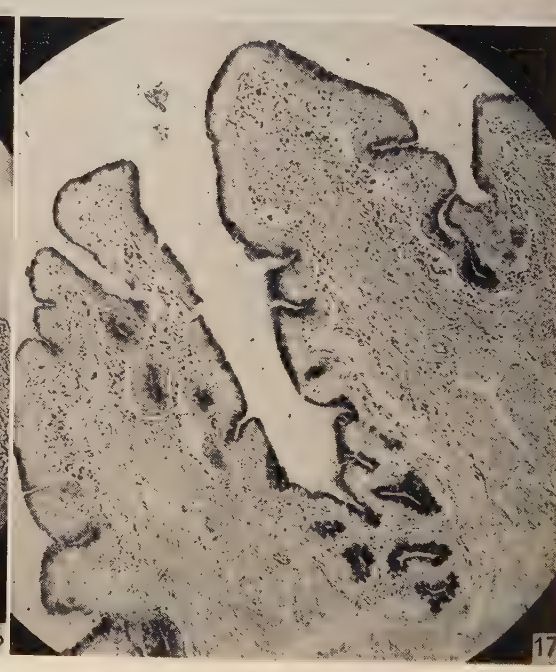


LAKE—THE MALE REPRODUCTIVE TRACT OF THE FOWL









LAKE —THE MALE REPRODUCTIVE TRACT OF THE FOWL

EXPLANATION OF PLATES

All sections stained with haematoxylin and eosin unless otherwise stated.

Plate 1

Fig. 1. Drawing of the reproductive tract and associated organs. The cloacal chamber is represented as having been slit in a cranial direction along both sides as the cock was lying on its back. The ventral portion was then turned on its vertical axis, so that the anterior end is facing back into the bird.

Fig. 2. Rough sketch of the testis and epididymal region.

Fig. 3. T.S. Cock testis showing thin t. albuginea and relatively large diameter tubules. No connective tissue septa are present which group tubules into lobules. $\times 50$.

Plate 2

Fig. 4. T.S. Cock testis showing numerous spermatozoa in each tubule. Spermatócytes are small. $\times 120$.

Fig. 5. T.S. One type of vasa efferentia epithelium showing the extrusion of large droplets of cell contents. Intra-epithelial glands are visible. $\times 600$.

Fig. 6. Sudan black B stain. Vasa efferentia epithelium showing lipid in the cells. A section of a satellite tubule is seen in the subepithelial tissue of one villus. $\times 500$.

Fig. 7. FSA stain. Another type of vasa efferentia epithelium showing positive granular material in cells. $\times 500$.

Fig. 8. Columnar epithelium of ductus epididymis. $\times 600$.

Fig. 9. T.S. Middle region of vas deferens. Note tortuous nature of duct and relatively wide lumen. $\times 40$.

Plate 3

Fig. 10. T.S. Distal, swollen region of vas deferens. Note increased thickness of fibrous tissue in the submucosa, and of the outer muscle layers. $\times 40$.

Fig. 11. Portion of epithelium of distal vas deferens to show extrusion of cellular apices. $\times 1200$.

Fig. 12. T.S. Ejaculatory duct in contracted state to show numerous small blood vessels in submucosa. $\times 80$.

Fig. 13. FSA stain. Epithelium on external surface of ejaculatory duct shows positive material in cells. $\times 80$.

Plate 4

Fig. 14. T.S. Vascular body in region of the base of ejaculatory duct near to ureter opening. Note goblet cells in epithelium, aggregations of lymphocytes surrounding the deep-lying glands and the vast blood sinuses in the sub-epithelial tissue. $\times 80$.

Fig. 15. FSA stain. Positive material in the glands of the vascular body. $\times 80$.

Fig. 16. Toluidine blue metachromasia. Section of vascular body to show acid mucopolysaccharide in gland cells. $\times 120$.

Fig. 17. Toluidine blue metachromasia. Part of lymph fold to show limited amount of acid mucopolysaccharide in epithelial cells. $\times 120$.

THE CYTOLOGY, HISTOCHEMISTRY AND ELECTRON MICROSCOPY OF THE GRANULAR CELLS OF THE METRIAL GLAND OF THE GRAVID RAT*

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Characteristic changes take place in the subplacental wall of the rat's uterus during gestation. The area involved, which includes the endometrium, myometrium and mesometrium at the placental site, has been called the 'metrial gland' by Selye & McKeown (1935). The most typical element of the 'gland' according to them is a 'granulated cell of maternal origin containing glycogen, eosinophilic, and sometimes also basophilic granules' which is located principally in the myometrial portion of this 'organ system'. Similar granular cells are present in experimentally induced deciduomas (Selye & McKeown, 1935; Asplund, Borell & Holmgren, 1940; Velardo, Dawson, Olson & Hisaw, 1953). Baker (1948) investigated the metrial gland of the rat by histochemical methods during pregnancy and lactation. According to him, the gland as it first develops is characterized by basophilic cells containing cytoplasmic ribonucleoprotein. Starting on about the 8th day of gestation, these cells gradually begin to lose their basophilia and become transformed into cells distinguished by the presence of conspicuous, eosinophilic granules. These cells persist until late in gestation when they decline and are gradually replaced by newly formed cells which are laden with lipides. Similar granular cells occur in the uterus of other rodents (mouse, guinea-pig) and rabbits.

The present investigation is concerned solely with the granular eosinophilic cells of the metrial gland. The cytoplasmic structure of these cells has been investigated in the present study by various cytological and histochemical methods, as well as by means of the electron microscope. The granules of the metrial cells have also been compared by the same techniques with the granules of the rat's eosinophilic leucocytes. Although the granules of both of these two types of cells are strongly eosinophilic, they exhibit characteristic histochemical differences which establish that they are different in chemical composition. They also differ markedly with respect to their structure as visualized by the electron microscope. The results also show that the procedures adopted can be utilized as a method for distinguishing different kinds of basic proteins.

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MATERIAL AND METHODS

The material was obtained from twelve female albino rats of the Sprague-Hawley strain maintained on a Purina chow diet. The rats were bred, and subsequently killed on the 13th, 15th and 18th days of pregnancy during the period when the granulated cells of the metrial gland are most plentiful.

Representative pieces of the chorio-allantoic placenta and the subplacental uterine wall were removed and placed in appropriate fixatives. The fixatives were: Zenker's acetic acid fluid, Rossman's ethanol-formalin-picric acid mixture, Orth's fluid, a 4 % aqueous solution of basic lead acetate, a mixture of 95 % methyl alcohol and 5 % glacial acetic acid, and a 10 % solution of buffered formalin. Excepting the formalin-fixed material of which frozen sections were prepared, the other blocks of tissue were embedded in paraffin, sectioned at 5μ, stained, cleared and mounted in clarite.

Sections of tissues fixed in Zenker's acetic acid fluid were stained by Gomori's chrome alum-haematoxylin and phloxine method, and by eosin and methylene blue. Two series of sections, fixed in the same way, were stained separately with methylene blue and with orange G at graded pH, from pH 8 to 3. Other series were stained similarly with eosin from pH 6 to 12, before and after extraction in pyridine, chloroform and ether-methanol, and after acetylation by pyridine in acetic anhydride (1:1) for 24 hr. at room temperature (Weiss, 1953). Other individual sections were stained for $\frac{1}{2}$ hr. in a 1 % aqueous solution of toluidine blue; control sections were placed in a solution of ribonuclease for 1 hr. at room temperature before staining them. Other sections were stained for protein-bound disulphide groups by the sulphydril and disulphide method of Barrnett & Seligman (1952), and for arginine by Baker's method (1947). NH_2 groups were demonstrated by the method of Weiss, Tsou & Seligman (1954), modified by the use of a more alkaline buffer (pH 9.1 against pH 8·5) and a staining time of 6 hr.

Sections of tissues fixed in either Rossman's or Orth's fluid were stained by McManus's periodic acid-Schiff procedure. Other sections were stained after immersing them first for 1 hr. in saliva to remove all glycogen.

Sections of the basic lead acetate-fixed material were stained for $\frac{1}{2}$ hr. in a 1 % aqueous solution of toluidine blue. Corresponding control sections were first exposed to a solution of ribonuclease for 1 hr. at room temperature before staining them.

Sections of the formalin-fixed material were cut on a freezing microtome at 5μ, stained in a saturated solution of Sudan black B in 70 % ethanol, and mounted in glycerogel.

For electron microscopy, metrial tissue was excised and cut into appropriately small pieces with a razor blade. The pieces were immediately fixed for 2 hr. in buffered osmic acid (Palade, 1952), washed in distilled water for $\frac{1}{2}$ hr. and dehydrated in 60 and 80 % ethanol for 1 hr. each. They were left overnight in two changes of 95 % ethanol. Next morning, they were placed in each of the following for 1 hr.: absolute alcohol, equal parts of absolute alcohol and *n*-butyl methacrylate, and two changes of pure *n*-butyl methacrylate. The tissues were embedded in gelatin capsules containing *n*-butyl methacrylate to which the catalyst 2:4-dichlorobenzoyl peroxide was added, and polymerization was carried out overnight in an oven at 45° F.

Ultrathin sections were cut with a glass knife on a Porter-Blum microtome, floated on a 20% acetone solution, picked up on a collodion-covered grid and examined and photographed in an RCA electron microscope, EMU-2E, without removing the methacrylate.

OBSERVATIONS

The development, topography and histology of the metrial gland of the rat have been described so extensively by previous investigators (cf. Selye & McKeown, 1935; Baker, 1948) that they need not be recapitulated here. Instead, for the orientation of the reader, we shall merely present two photographs to illustrate the location and general nature of the gland. Pl. 1, fig. 1, is of a transverse section of a rat's pregnant uterus on the 13th day of gestation. The placenta (*pl*), endometrial decidua (*de*) and myometrium (*my*) are visible. A rectangle encloses a field embracing a small portion of the decidua and a somewhat larger area of the myometrium. Pl. 1, fig. 2, shows the area in the rectangle enlarged, revealing numerous granular cells with their acidophilic granules differentiated by phloxine. The decidua in the upper part of the field is separated from the myometrium in the lower portion of the picture by an oblique line of interstitial material. The granular cells are far more abundant in the myometrial than in the decidual (endometrial) zone of the 'metrial gland'. However, in fig. 2 at the junction of the two zones they are visible in both regions. These granular cells are most abundant between the 12th and 18th days of gestation.

The granular cells are relatively large, mononucleate or binucleate elements with a conspicuous Golgi region situated to one side of the nucleus or between the two nuclei (Pl. 2). The granules are usually clustered immediately around the Golgi region in the centre of the cells. The extensive peripheral portion of the cytoplasm is in most instances clear and vacuolated with a variably distinct cell membrane separating it from the surrounding intercellular matrix.

The cell granules have distinctive characteristics. They are acidophilic, as revealed by their intense staining with phloxine (Pl. 1, fig. 2), eosin (Pl. 2, fig. 6) and orange G (Pl. 2, fig. 8). Investigation of their dye-binding capacity for eosin reveals intense staining of the granules at pH 10·3 with an estimated value of 3+ and a sharp decline in staining at pH 11·5 to an estimated value of only 1+. Following acetylation by pyridine in acetic anhydride (1:1) for 24 hr. at room temperature, the granules fail to bind eosin or they bind it at most to a very slight degree. Following extraction by pyridine, chloroform, or ether methanol, the eosinophilic staining remains undiminished. The granules are highly reactive for protein-bound amino groups (Pl. 2, fig. 11) and protein-bound disulphide groups (Pl. 2, fig. 10), but do not react with Baker's method for arginine.

The chemical properties of the specific granules of the metrial cells differ from those of eosinophilic leucocytes within placental and uterine blood vessels. In contrast to the granules of the metrial cells, those of the eosinophilic leucocytes show intense acid dye-binding at pH 11·5, which is unimpaired by acetylation; they give a strongly positive reaction with Baker's method for arginine and remain unstained by the procedure for NH_2 groups. The significance of these reactions will be interpreted in the discussion with respect to the chemical nature of the granules in the metrial cells and eosinophilic leucocytes.

Stained with toluidine blue at approximately neutral pH, the granules of the metrial cells are metachromatic after fixation in either basic lead acetate or Zenker's acetic acid mixture (Pl. 2, figs. 12, 13). Following immersion of the sections in ribonuclease previous to staining them, this metachromasia is undiminished (Pl. 2, fig. 14). Stained with methylene blue at pH 8 and below, the granules are unstained (Pl. 2, fig. 9). The granules are intensely reactive with the periodic acid-Schiff stain (Pl. 2, fig. 7). With Sudan black B they are quite unstained, the only visible reaction in the cells consisting of a faint coloration of the Golgi region.

The cells reveal a small to moderate degree of cytoplasmic basophilia when sections are stained with methylene blue. The basophilic substance is visible in clumps and strands located variously between the granules and peripheral vacuoles of the cells (Pl. 2, figs. 6, 9 and 12). The basophilia is almost completely abolished by pre-treatment of the sections with ribonuclease, a result indicating that the substance consists of ribonucleoprotein (cf. Pl. 2, figs. 12, 14). The basophilic substance stains with methylene blue at relatively low pH, ceasing around pH 4·5, a result characteristic of nucleoproteins which are acidic.

The large vacuoles in the peripheral cytoplasm of the cells contain glycogen, as can be demonstrated by appropriate means. Pl. 1, figs. 3 and 4, illustrate the total degree of staining of the granular cells by the periodic acid-Schiff procedure, whereas Pl. 1, fig. 5 and Pl. 2, fig. 7, illustrate the residual staining of the cells following exposure of the sections to saliva. The difference in amount of staining before and after treatment with saliva represents glycogen present in the large peripheral vacuoles. The intense residual periodic acid-Schiff reaction is confined entirely to a carbohydrate present in the granules (Pl. 1, fig. 5; Pl. 2, fig. 7), which is resistant to digestion by saliva. In the preparation shown in Pl. 1, fig. 3, it will be noted that the glycogen has drifted to one side of the cells as a result of the penetration of the fixative from the opposite side. There appears to be relatively more glycogen at 18 days (Pl. 1, fig. 4) than at 15 days (Pl. 1, fig. 3).

Examination of the metrial gland in the electron microscope confirms and amplifies some of the cytological and histochemical observations. The findings are illustrated in Pls. 3 and 4. In the electron micrographs, four distinctive organelles are visible in the cytoplasm, namely specific granules, the Golgi apparatus, mitochondria and endoplasmic reticulum (Pl. 3, fig. 15; Pl. 4, figs. 17, 18).

The specific granules (Pl. 4, fig. 17, *Gr*) which correspond in distribution and number to the acidic material are surrounded by a loosely fitting membrane (Pl. 3, fig. 15; Pl. 4, figs. 17, 18). The smaller the granule, the larger is the lacunar space between it and the surrounding envelope. The granules vary in density and some contain several clear vacuolar spaces within them. The surfaces of the granules may be smooth or irregular. The irregularities are due to minute vesicles which, in places, appear to be coalescing with the substance of the granules (Pl. 4, fig. 17, *V*). In some instances, these small vesicles are regularly dispersed in the lacunar space between the granule and its enveloping membrane (Pl. 4, fig. 18, *V*).

The endoplasmic reticulum in the metrial cells appears as double membranes or as flattened vesicles connected with lacunar spaces (Pl. 4, fig. 18, *R*). Palade's granules are present on the outer aspects of these membranes and some are randomly scattered throughout the cytoplasm.

The metrial cells also contain numerous vesicles of different sizes and shapes which are enclosed by double membranes. These structures are concentrated for the most part near the nucleus in the Golgi region (Pl. 4, fig. 17). The Golgi complex, clearly defined by an aggregation of flattened vesicles, is typically located between the granules and the nucleus (Pl. 3, fig. 15; Pl. 4, fig. 17, *G*) in the same position as it is seen with the light microscope.

A few short oval mitochondria (Pl. 4, figs. 17, 18, *M*) appear randomly distributed between the granules. They have a clear or vacuolated matrix and sharply defined cristae.

In addition to these organelles, the cytoplasm contains diffusely scattered, greyish flocculent material believed to represent the glycogen identified in preparations stained by the periodic acid-Schiff reagent.

The granules of the rat's eosinophilic leucocytes are quite different from those of the metrial cells as seen with the electron microscope. The leucocytic granules are distinguished by a sharply demarcated, dense, meridional band (Pl. 3, fig. 16). Moreover, there is no space and loose membranous envelope associated with the granules, and consequently there are no small vesicles associated with their surfaces. The marked differences between the two types of granules may be compared in Pl. 3, fig. 16, and Pl. 4, figs. 17, 18.

DISCUSSION

The most interesting question raised by the present findings concerns the nature of the protein composing the acidophilic granules of the metrial cells. The substance of the granules is quite basic since it binds an acid dye, such as eosin, up to relatively high pH. The observation that eosin is intensely bound up to pH 10·3, but that at pH 11·5 binding has practically ceased, suggests that the basic substance is composed of ϵ-NH_2 groups of lysine. These groups, while quite basic, are not as basic as the guanidonium groups of arginine or the tertiary and quaternary amines which bind acid dyes as high as pH 12 (Cohn & Edsall, 1943). The failure to bind eosin following acetylation indicates that NH_2 groups or hydrogenated amines are responsible for the observed dye-binding, because the guanidonium groups of arginine and the tertiary and quaternary amines, having no replaceable hydrogen, cannot be acetylated under the prevailing conditions (Weiss, 1953). Additional support for this conclusion is seen in the persistence of staining after extraction by fat solvents, because, unlike the protein-bound ϵ-NH_2 of lysine, tertiary and quaternary amines which are typically associated with phospholipid would be dissolved. The negative reaction with Baker's method for arginine also provides evidence that the eosinophilia is not due to the guanidonium groups of arginine. Finally, the strong reaction with the method for NH_2 groups also supports the thesis that those groups are responsible for the eosinophilia. From these data, it is concluded that the eosinophilia of the granules of the metrial cells is due mainly to the ϵ-NH_2 groups of lysine.

The histochemical methods adopted here provide a means of differentiating and distinguishing different basic proteins, as is illustrated in the following table which compares, in the rat, some of the histochemical reactions of the granules of the metrial cells with those of eosinophilic leucocytes (Table 1).

From these chemical differences, it is concluded that the granules of the eosinophilic leucocytes are composed of a basic protein which is rich in the amino-acid arginine, instead of lysine which characterizes the metrial cells. These comparisons illustrate the possibility of distinguishing basic proteins from one another by histochemical means. The validity of the conclusion that the metrial cell granules differ in composition from eosinophilic leucocyte granules, is also borne out by their markedly different morphology in the electron microscope (cf. Pl. 3, fig. 16; Pl. 4, figs. 17, 18).

Table 1. *Comparisons of some histochemical reactions of the granules of the metrial cell and of eosinophilic leucocytes*

Method	Metrial cell	Eosinophilic leucocyte
Acid dye-binding (with eosin)	Intense at pH 10·3, slight at pH 11·5	Intense at pH 11·5
Acid dye-binding after acetylation	Unstained	Stained
Reaction for arginine	Negative	Strong positive
Reaction for NH_2 groups	Strong positive	Negative

Besides the evidence advanced that the granules of the metrial cells contain lysine rather than arginine, the intense staining for disulphide groups by Barrnett & Seligman's sulphydril-disulphide method suggests that the substance of the granules is also rich in cystine.

Another point in need of brief clarification is the assertion by Selye & McKeown (1935) that the granulated cells contain eosinophilic as well as basophilic granules in one and the same cell. The former, they say, stain with fuchsin or eosin, while the latter stain with aniline blue. This is a misconception based on the false assumption that aniline blue is a basic dye and hence stains acidic or basophilic granules. Actually, aniline blue is an acid dye which has an affinity for basic or alkaline substances. The staining of some granules by aniline blue in contrast to the majority which was eosinophilic as reported by Selye & McKeown, signifies merely that the substance of individual granules varies somewhat with respect to its isoelectric point and consequently its affinity for individual acid dyes.

Although it has been known for a long time that the granulated metrial cells of the pregnant rat's uterus contain glycogen (cf. Selye & McKeown, 1935; Bridgman, 1948), ours is the first detailed description of the staining of these cells by the periodic acid-Schiff method. By this technique it is apparent that, in addition to a relatively large amount of glycogen which is diffusely distributed in the cytoplasm and can be removed by treatment with saliva, there is a pronounced saliva-resistant, residual staining of the specific granules (Pl. 1, fig. 5; Pl. 2, fig. 7) indicating the presence in them of a glycoprotein or mucopolysaccharide.

The possibility of a carbohydrate being conjugated with the protein of the cell granules leads us to a consideration of the significance of the metachromasia of the granules. Asplund *et al.* (1940) first called attention to the fact that the specific eosinophilic granules of the metrial cells of the rat, mouse and rabbit stain metachromatically in an aqueous solution of toluidine blue, particularly after fixation in a 4% aqueous solution of basic lead acetate. Metachromatic staining after this particular procedure they accepted as proof of the presence of a sulphated muco-

polysaccharide. Furthermore, from observations of the influence of acid, alkaline and physiological salt solutions upon the staining of the granules of the metrial cells they concluded that the granules contain a sulphated mucopolysaccharide of a low degree of esterification.

In recent years the thesis of Lison (1936) that all metachromatic staining by thiazine dyes should be attributed to the presence of sulphated acid mucopolysaccharides has been questioned (cf. Gomori, 1952; Pearse, 1953). According to Pearse, relatively alcohol-resistant metachromasia in paraffin sections is most likely to be due to sulphate esters. However, metachromasia of lesser intensities can be caused by polymerized carbohydrates by virtue of their carboxyl groups (Michaelis, 1947) or by phosphate-containing compounds. Ribose nucleic acid, for example, sometimes shows metachromasia in paraffin sections (Wislocki, Bunting & Dempsey, 1947), which can be prevented by removing the nucleic acid with ribonuclease. However, our failure to prevent the staining of the granules of the metrial cells by ribonuclease indicates that their metachromasia is not associated with ribose nucleic acid. That the substance producing the metachromasia must be relatively feebly acidic is indicated by its failure to bind methylene blue even at pH 8.

It may be presumptuous to offer any explanation of the intense acidophilia of the granules combined with their observed metachromasia. Nevertheless, the speculation is tentatively advanced that one is dealing here with a strongly alkaline protein conjugated with mildly acidic prosthetic groups. If that assumption is correct, the strongly alkaline protein would account for the intense affinity of the granules for acid dyes and their weak reaction towards methylene blue, whereas the acidic prosthetic groups would have to be held specifically responsible for the metachromatic staining with toluidine blue. The intense reaction of the granules with the periodic acid-Schiff reagent following removal of all glycogen should probably be attributed to a mucopolysaccharide representing possibly the same prosthetic groups responsible for the metachromatic staining.

The electron micrographs of the metrial cells reveal a Golgi complex close to the nucleus, specific cell granules, mitochondria and some endoplasmic reticulum.

The specific granules are peculiar in many respects, including the presence of numerous minute vesicles in the space between the granule and the capsule which loosely surrounds it. The association of these vesicles with the smaller granules and their apparent incorporation into the granules suggest that the vesicles may be related to the formation and growth of the granules of the metrial cells.

In some places the membrane which encloses the granule and the perigranular space appears to be related to endoplasmic reticulum. In Pl. 4, fig. 18, the membrane surrounding the granule and irregular space marked '*V*' appears to be continuous at its lower border with a strand of endoplasmic reticulum. Other similar appearances have been noted. If this speculation is correct, then the outer membrane and the lacunar space of each granule would be equivalent to a dilated cisterna of endoplasmic reticulum. The cytological observation made by Baker (1948) that cytoplasmic basophilia disappears inversely with the development of the acidophilic granules would seem to support such an assumption. These considerations suggest studying the origin of the metrial cells and the mechanism of formation of their granules by observing earlier stages than those examined here.

Brief comment and speculation may be welcome regarding the possible function of the granular cells of the 'metrial gland' of rodents and rabbits. In recent years the formation of relaxin, steroid hormones and histamine has been variously ascribed in some of these species to the activity of the decidually transformed uterine wall in pseudopregnancy or pregnancy. Thus, relaxin has been found in the uterus and placenta of the guinea-pig and rabbit (Hisaw & Zarrow, 1950), as well as in the uterus and maternal placenta of the rat in pseudopregnancy and pregnancy respectively (Zarrow, 1956). Frieden & Hisaw (1953) characterize relaxin as a simple protein which according to Kraintz (1951) contains lysine and cysteic acid (representing cystine and cysteine) among several amino-acids, whereas arginine is absent. The resemblance of relaxin in these respects to the protein of the granules of the metrial cells of the rat, as observed in the present investigation, is apparent.

The granular cells in question would not seem to be implicated in any possible steroid synthesis since they do not contain lipid material, aside from a slight staining of their mitochondria and elements of the Golgi complex with Sudan black B. With respect, however, to the possible synthesis of steroid compounds by the metrial gland, observations by Baker (1948) are of interest. He describes lipid-laden cells in the rat's metrial gland which gradually replace the granular cells and of which he remarks that, judged by their histochemical reactions, they may contribute to steroid metabolism late in gestation. These cells are, however, totally different from the granular cells described in the present study.

With respect to histamine, Shelesnyak (1952, 1954) observed that antihistaminic drugs suppress the development of the decidual reaction in pseudo-pregnant rats, from which he postulated and discussed the possibility 'that histamine release may be the operative mechanism in initiating natural placentation'. These observations offer the slight connexion with the present study that histamine is regarded as being produced by mast cells (Riley & West, 1953; Fawcett, 1954) which are characterized by the presence of strongly basophilic cytoplasmic granules which are intensely metachromatic. This recalls to mind the metachromatic attribute of the eosinophilic granules in the metrial cells discussed above.

SUMMARY

An account is given of the cytology, histochemistry and electron microscopy of the granular cells of the metrial gland of the gravid rat. The granules of these cells bind an acid dye (eosin) intensely at pH 10·3 and only slightly at pH 11·5, do not stain after acetylation, do not give a reaction for arginine and give a strongly positive reaction for NH_2 groups. From these data it is concluded that the eosinophilia of the granules is due mainly to the ϵ-NH_2 groups of lysine.

The granules also exhibit moderate metachromasia after staining with toluidine blue and stain intensely with the periodic acid-Schiff reaction. These staining properties are discussed, and it is concluded that they pertain to a mucopolysaccharide conjugated with the alkaline protein.

In electron micrographs the cytoplasm of these cells is observed to contain numerous specific granules, mitochondria, endoplasmic reticulum and glycogen. The specific granules are electron-dense, spherical objects surrounded by an

encapsulating membrane and a variously extensive, intervening space which contains minute vacuoles.

The granules of the metrial cells were compared with the granules of the eosinophilic leucocytes of the rat. In sharp contrast to the former, the eosinophilic granules of the leucocytes bind acid dyes intensely at pH 11·5, bind acid dyes after acetylation, react strongly for arginine and do not give a reaction for NH_2 groups. From these chemical differences it is concluded that the granules of the eosinophilic leucocytes consist of a basic protein rich in the amino-acid arginine, instead of lysine which characterizes the granules of the metrial cells. The histochemical procedures adopted in comparing these two types of granules provide a means of distinguishing different basic proteins. That the two kinds of granules differ is shown also in electron micrographs, where the granules of the eosinophilic leucocytes are observed to possess a wide, meridional, electron-dense band and not to have a perigranular space or capsule.

With respect to the possible function of the rat's metrial cells, the suggestion is offered that their basic protein granules which contain lysine but little or no arginine might be related to the presence of relaxin. Recent chemical evidence is cited which indicates that relaxin is a simple protein having lysine but not arginine amongst its amino-acid constituents.

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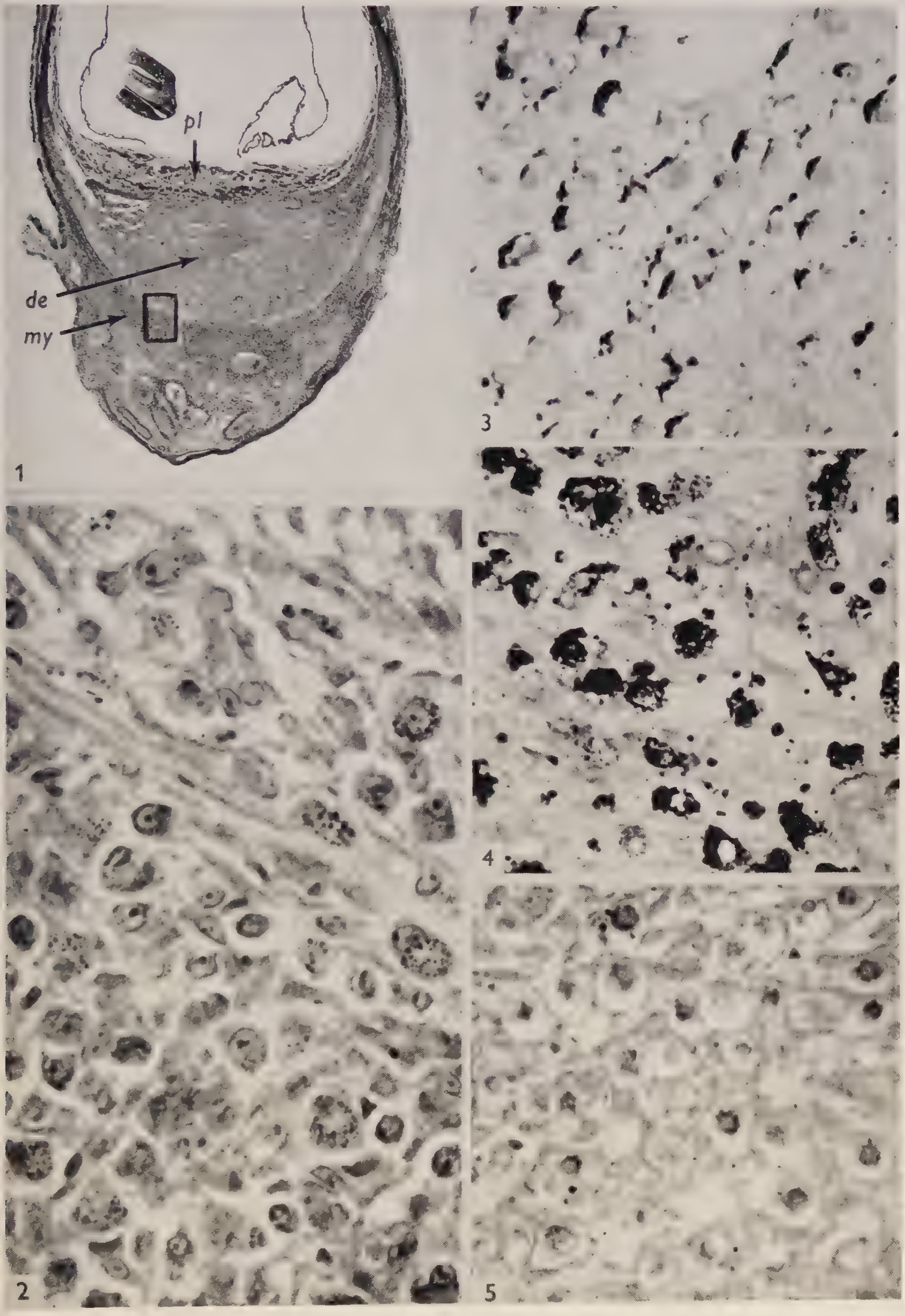

WISLOCKI AND OTHERS—GRANULAR CELLS OF RAT'S METRIAL GLAND

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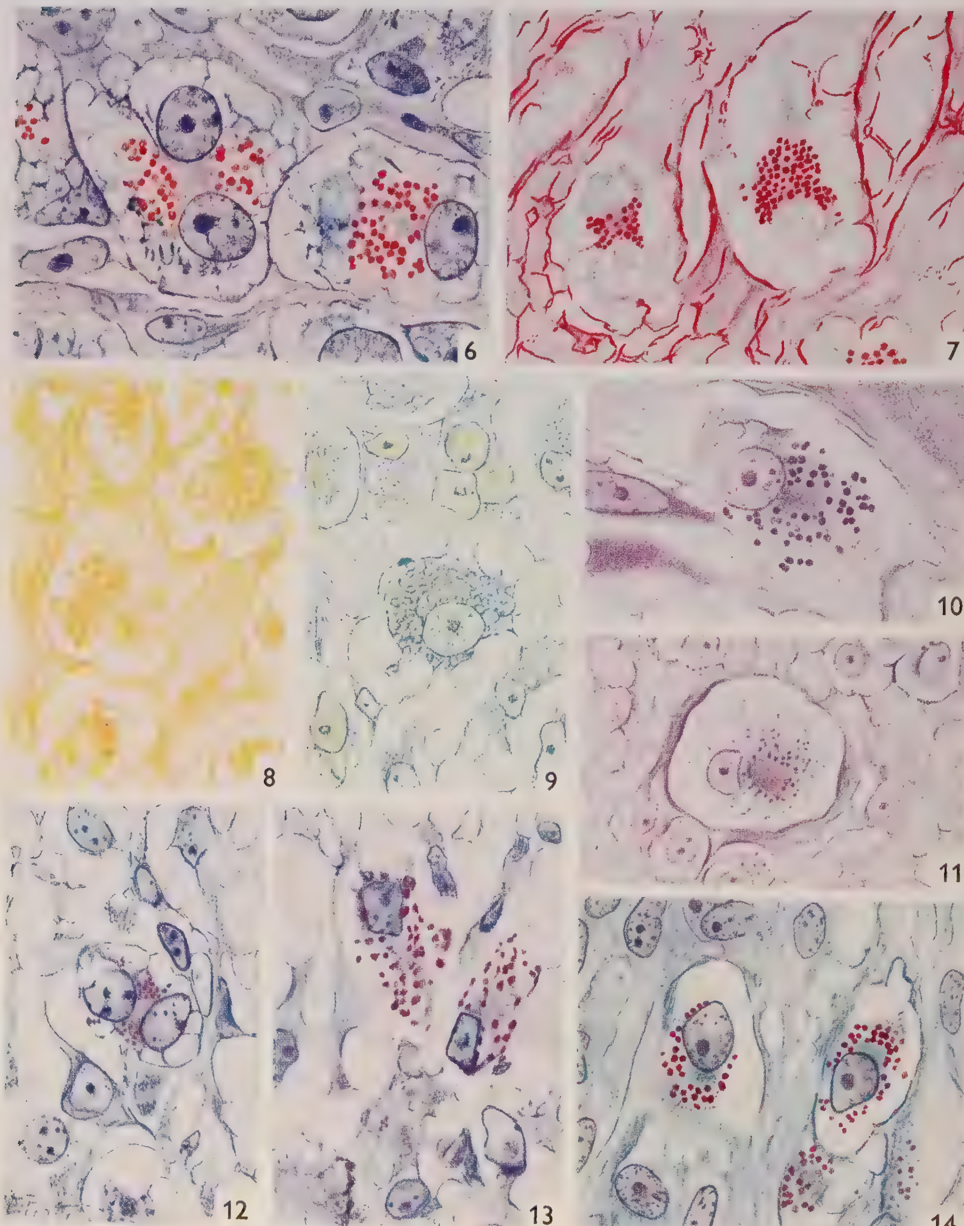

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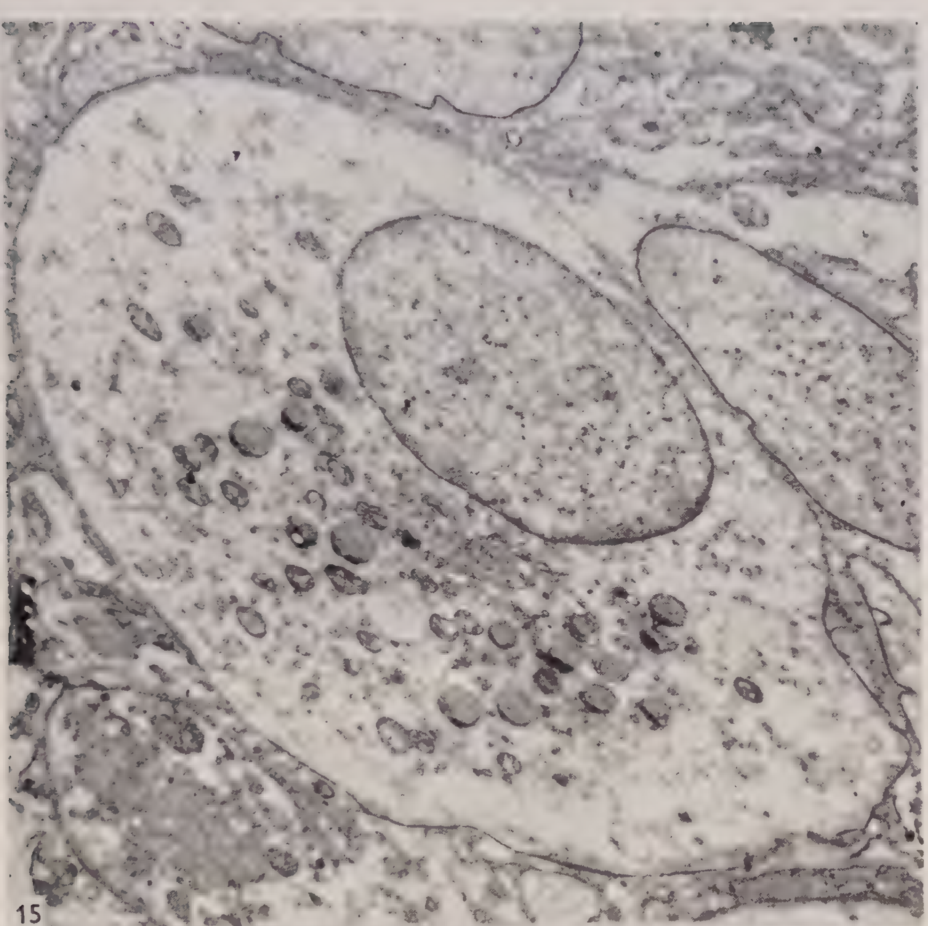

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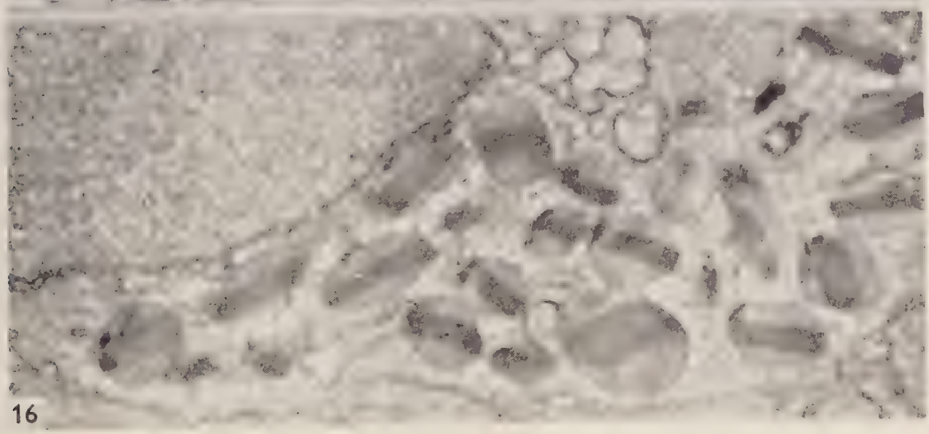

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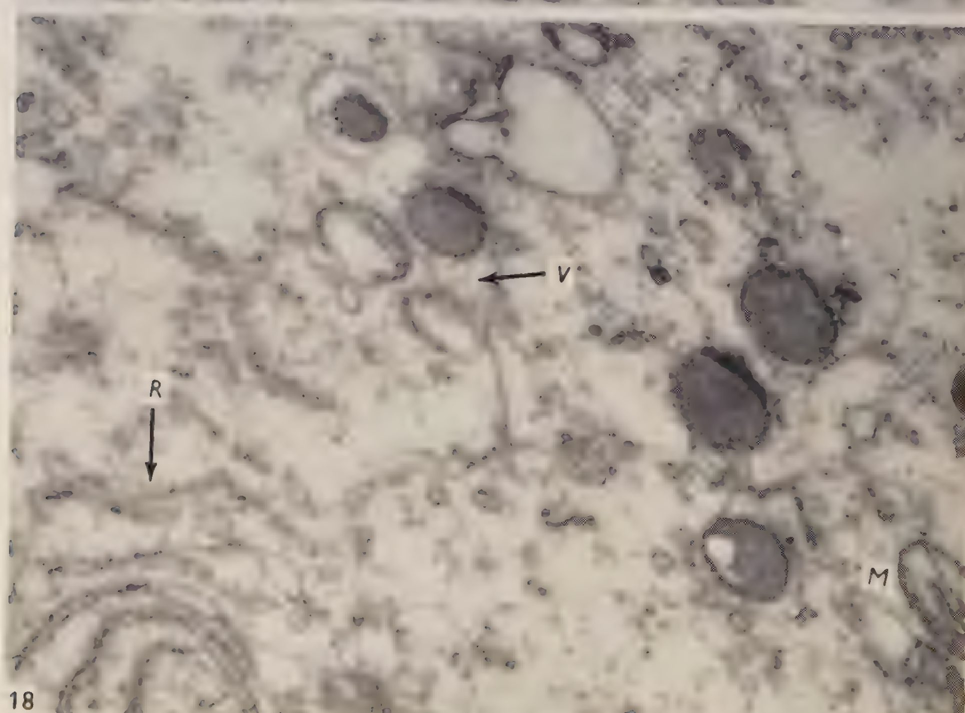
WISLOCKI AND OTHERS—GRANULAR CELLS OF RAT'S METRIAL GLAND



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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Histological section of a pregnant rat's uterus at 13 days of gestation. *pl*, the chorio-allantoic placenta; *de*, the endometrial decidua; *my*, the myometrium. The area contained in the rectangle is shown at higher magnification in fig. 2. Chrome alum-haematoxylin and phloxine stain. × 13.

Fig. 2. A portion of the endometrial decidua and myometrium (area contained in the rectangle in fig. 1), illustrating the granular cells of the metrial gland which are characteristic of the second half of gestation. Chrome alum-haematoxylin and phloxine. × 600.

Fig. 3. The metrial gland of a rat on the 15th day of pregnancy. The granular cells which contain glycogen have been stained by the periodic acid-Schiff reagent. The deeply stained glycogen has drifted to the side of the cells opposite to the direction of penetration of the fixative. Rossman's alcohol-formalin-picric acid fixative. × 480.

Fig. 4. The metrial gland on the 18th day of pregnancy, stained with the periodic acid-Schiff reagent. Same fixative as the preceding. × 480.

Fig. 5. The metrial gland on the 13th day of pregnancy. The section was exposed to saliva to remove all glycogen before staining with the periodic acid-Schiff reagent. An intense residual reaction due to a saliva-resistant substance regarded as a mucopolysaccharide or glyco-protein, is visible in the granules in the vicinity of the nucleus. The detailed appearance of two such cells is illustrated in Pl. 2, fig. 7. × 480.

PLATE 2

The figures on this plate all represent granular cells of the rat's metrial gland, drawn with a × 7 ocular and × 90 objective, excepting fig. 6 which was drawn with a × 10 ocular and figure 11 with a × 60 objective.

Fig. 6. Two binucleate granular cells from the 15th day of gestation, stained with eosin and methylene blue. Zenker's acetic acid fixative. Intensely eosinophilic granules surround the Golgi region, whereas the peripheral cytoplasm contains basophilic material representing ribonucleoprotein, and vacuoles.

Fig. 7. Two granular cells from the 18th day of gestation, illustrating the strong affinity of the granules for the periodic acid-Schiff stain. The section was exposed to saliva to remove glycogen prior to staining it. Rossman's fixative.

Fig. 8. Granular cells stained with orange G at pH 5·13, on the 15th day of gestation. Zenker's acetic acid fixative.

Fig. 9. A section similar to the preceding one, stained with methylene blue at pH 5·13. Note that the acidophilic granules have no affinity at this pH for methylene blue, a basic dye, whereas the dye has an affinity for cytoplasmic basophilic substance surrounding the granules. The latter substance, which is also visible in the cells in fig. 6, can be abolished by ribonuclease.

Fig. 10. A granular cell stained by the method of Weiss, Tsou and Seligman for the demonstration of protein-bound amino groups, showing the intense reaction of the cell granules.

Fig. 11. A similar cell, at lower magnification, illustrating the presence of protein-bound disulphide groups in the cell granules.

Fig. 12. A binucleate metrial cell, showing the metachromasia of its granules following staining with toluidine blue. Observe that the granules surround the Golgi region. Zenker's acetic acid fixative.

Fig. 13. Two granular cells stained with toluidine blue, following fixation in a 4 % aqueous solution of basic lead acetate. This is a technique recommended by Holmgren & Wilander (1937), following which they observed metachromatic staining of the granules of the metrial cells in a variety of rodents. The metachromatic staining depicted in the drawing confirms their observation.

Fig. 14. A section similar to the one in fig. 13, revealing the metachromasia of the granules following exposure of the section to ribonuclease.

PLATE 3

Fig. 15. An electron micrograph of a granulated cell of the rat's metrial gland, illustrating the nucleus and the smooth plasma membrane of the cell. The cytoplasm contains a number of electron-dense granules and some mitochondria, as well as a dense Golgi complex situated near the nucleus. The greyish flocculence throughout the cytoplasm is regarded as representing glycogen. The granules in this cell correspond in number, size and distribution to the specific acidophilic granules encountered in the light microscope. × 5,818.

Fig. 16. An electron micrograph of a portion of a rat's eosinophilic leucocyte. Part of the cell nucleus is present in the upper part of the figure. The characteristic eosinophilic granules present in the cell cytoplasm possess a distinct meridional band. Compare with Pl. 4, figs. 17, 18. × 32,000.

PLATE 4

Fig. 17. A portion of the cytoplasm of a granular metrial gland cell at higher magnification. The Golgi apparatus (*G*) is visible surrounded by five electron-dense, encapsulated granules (*Gr*) and several mitochondria (*M*). The smaller granules are usually surrounded by a space which contains minute vacuoles (*V*). × 30,600.

Fig. 18. Another portion of the cytoplasm of a granular cell revealing a number of granules, one of which is surrounded by an irregular capsule and space which contains minute vacuoles (*V*). Sheets of endoplasmic reticulum (*R*) are visible in the lower left-hand corner of the electron micrograph. Two mitochondria (*M*) are shown. Amorphous background material is believed to represent glycogen. The organelles seen in the electron micrograph (Pls. 3 and 4) should be compared with the cytological structures revealed at lower magnification in the light microscope (Pl. 2). × 16,525.

E. B. JAMIESON, M.D.

(*Facing p.* 141)

IN MEMORIAM

E. B. JAMIESON, M.D.

Dr E. B. Jamieson, whose whole academic life was spent in the University of Edinburgh, died at Edinburgh on 16 August 1956, a few months after he had attained the age of 80 years. He was so well known to many generations of Edinburgh students by his constant interest in their welfare, both before and after graduation, and to many others all over the world by his popular anatomical publications, that the news of his death has been received with a sense of personal loss which has stimulated numerous tributes in the Medical Press.

Born in Shetland in 1876, Edward Bald Jamieson was the fifth son of Robert Jamieson, schoolmaster of Sandness, other members of whose family also distinguished themselves academically and in the medical profession. His eldest brother, Francis, was Lecturer in Humanity in the University of Edinburgh and then H.M. Chief Inspector of Schools for Scotland; another elder brother was John Kay, Professor of Anatomy at Leeds and Trinity College, University of Dublin, whose Obituary Notice appeared in the *Journal of Anatomy* (**83**, 47) in 1949; and his younger brother is the well-known and distinguished practitioner of Nelson, New Zealand, Dr J. P. S. Jamieson. Like his brothers, E. B. Jamieson attended his father's Sandness Madras School before proceeding to the University of Edinburgh.

After graduating M.B., Ch.B. in 1900, he at once joined the staff of the Department of Anatomy under Sir William Turner; and in 1909, at the time of the appointment of Arthur Robinson to succeed D. J. Cunningham as professor, Jamieson succeeded David Waterston as Senior Lecturer, a post he was to occupy for no less than 36 years. Normally he would have retired at the age of 65 in 1941, but he was continued in office until near the end of the War, having then served continuously in the Department, during the occupation of the Chair by four professors, for nearly 45 years.

Jamieson's original contributions to anatomy were not numerous, as his main interest lay in teaching. In 1903 he had published 'A description of some Anomalies in Nerves arising from the Lumbar Plexus of a Foetus, and of the Bilaminar Musculus Pectineus in the same Foetus; etc.', but thereafter his chief original work was the development of ordinary dissecting methods for the display of the gross structure of the brain. At the First International Congress of Anatomy at Geneva in 1905, he had given a 'Demonstration of Various Tracts of Fibres and Masses of Grey Matter of the Brain, displayed by ordinary dissection'; and this was the subject of his M.D. thesis—entitled 'A Description of Several Series of Unusual Dissections of the Human Brain'—for which he received his University's Gold Medal in the following year. Formal publications on the same theme, both in the *Journal of Anatomy and Physiology*, were 'The Means of displaying by Ordinary Dissection the Larger Tracts of White Matter of the Brain in their Continuity' (1909, **43**, 225–234) and 'The Arrangement of the Fibres of the Middle Cerebellar Peduncle by Dissection'

(1910, 44, 234–240). After his retirement at the end of the War, he devoted many hours to the preparation of a series of brain-dissections which, in response to the appeal for assistance in replacing collections destroyed by enemy action, he presented to the Museum of the Royal College of Surgeons of England.

But, as has been indicated, Jamieson was very assiduous in his teaching duties, and his chief contributions to anatomical literature have been in the form of text-books and atlases. His daily Lecture-Demonstration began the work of the Department for many years at 9 a.m., and a great proportion of his time was spent in the dissecting room for which he was responsible. The blackboard drawings with which he illustrated his lectures were gathered together and published between 1934 and 1936 as *Illustrations of Regional Anatomy*. This loose-leaf sectional atlas—the work by which he is probably best known—enjoyed great popularity, a reprint of the 7th edition being called for in 1951. A shorter form as *Illustrations of Anatomy for Nurses* appeared in 1946 and reached a 3rd edition in 1950.

Another popular work was *A Companion to Manuals of Practical Anatomy*; it was published in 1913 and reached a 7th edition in 1950. He wrote *Surface Anatomy* (jointly with Arthur Robinson) in 1928, and provided a 2nd edition of *Dixon's Manual of Human Osteology* in 1937. He wrote the Section on 'Osteology' in the 5th to the 8th editions of *Cunningham's Text-book of Anatomy*, and he was joint editor of the 7th and 8th (1937/1943). He was jointly responsible also for the 9th and 10th editions of *Cunningham's Manual of Practical Anatomy* (1935/1940).

Jamieson had taken a great and classically informed interest in the discussions of anatomical nomenclature which began soon after the introduction of the BNA, of which he published an account in 1917. He was, with A. F. Dixon and T. B. Johnston, a member of the Committee appointed by the Anatomical Society in 1928 to consider its revision, and he took a leading part in preparing their Interim Report, presented to the Society with commendable promptitude in the following year, and in the preparation of their Final Report adopted by the Society at Birmingham in 1933.

No Notice of E. B. Jamieson would be complete without reference to the impact of his personality on generations of Edinburgh students and on generations also of University Staff. Although his funeral took place in the middle of the summer vacation, many of his old students were present and there was a representative attendance of the staff. With his Norse origin of which he was proud, he was a man of striking appearance, accentuated by his habit of wearing a close-fitting black skull-cap not only during his working hours. His strong personal characteristics impressed all who made his acquaintance and not least students at their first interviews. But, in spite of his austere manner and frequent disciplinary homilies—with particular reference to 'idleness'—students soon learned to recognize in Dr Jamieson a wise counsellor and friend. Indeed, the Faculty of Medicine owed a great debt to him for the way in which he acted for many years, before official arrangements were made, as unofficial and informal 'tutor' to many generations of medical students. No teacher can ever have had closer relations with his students, and his exact memory of their individual appearance, their names and initials, year of entry, home address and often their parentage was a perpetual wonder and early became a tradition. Jamieson himself admitted that he might sometimes be at

fault in respect of some of those who entered in the crowded years after the 1914-18 War; but even to the end he was able with little hesitation to give such particulars of many who had been students more than 40 years before.

E. B. Jamieson's name will be commemorated in the University of Edinburgh by his generous gift on his retirement of a substantial fund for the award of prizes to final year students for knowledge of Regional Anatomy. This fund is now to be annually augmented by royalties on his books that may still accrue, which by his will he has assigned to the University after his death.

He joined the Anatomical Society in 1905, was elected a life member in 1950 and at his death was its senior member. J.C.B.

[The plate was made from a copyright photograph of Dr Jamieson taken by A. Swan Watson, Edinburgh.]

REVIEWS

The Human Figure in Motion. By EADWEARD MUYBRIDGE. ($7\frac{7}{8} \times 10\frac{3}{4}$ in.; $10.00.) Dover Publications Inc. Available in England through the Victory Publishing Company, 41–45 Neal Street, London, W.C. 2.

This is a unique, remarkable and interesting book of 195 plates each occupying two opposing pages and each containing up to 36 figures, all told a few thousand photographs of consecutive attitudes of nude men, women and children engaged in various activities.

Apart from walking, running, jumping, dancing, crawling on all fours, walking up and down stairs and coming down stairs backwards, there are the more vigorous actions of somersaulting, rowing, hurling weights, boxing, wrestling, hand balancing, fencing, using a lawn roller, hammering at an anvil, brick laying, hod carrying up a ladder, carrying a pail in one hand and carrying a pail in each hand, as well as such graceful exercises as carrying a basket on the head, carrying a fan in one hand and a bouquet in the other, picking up and throwing a scarf over the shoulders while walking, and pulling on a stocking. There are some unexpected sequences such as a woman spanking a child, walking with crutches, and the progression of a legless boy getting into and out of a chair.

The above enumeration serves to indicate the multiplicity of studies.

These are valuable figures for all who may be concerned with the poise of the limbs, trunk and head in individual and also in consecutive attitudes, and with the joint movements placing the muscle groups involved in the best position for their most powerful action. It may be that such a series as a woman getting into bed, reclining in a hammock or leaning back in a chair while smoking a cigarette are of more interest to the artist than the anatomist. In general the reproduction is not clear enough to indicate the surface contour of individual muscles but this is not a serious detraction when poise is the main purpose of the illustrations.

This publication has certain historical interest. It is a selection from *Animals in Motion*, a total of 781 plates from photographs by Muybridge some 70 years ago. This selection was made by Alex Domonkos of the Art School of Westport, Connecticut, and by Dr Wallace Green, Orthopaedic Surgeon, the historical introduction by Prof. Robert Taft, University of Kansas.

It is interesting to note that Prof. Sir James Gray in his recent article, 'Muscular Activity During Locomotion', *British Medical Bulletin*, **12**, no. 3, September 1956, reproduces Muybridge's pictures of a galloping horse and of a man running, with the reference E. Muybridge (1899), *Animals in Motion: An Electro-photographic Investigation of Consecutive Phases of Animal Progressive Movements*, Chapman and Hall, London. R. D. LOCKHART

The Biochemistry and Physiology of Bone. Edited by GEOFFREY H. BOURNE. (Pp. xiv + 875; 131 text-figures; 44 plates, 1 coloured, with 243 figs.; 40 tables; $9\frac{1}{4} \times 6\frac{1}{4}$ in.; $20.00, £7. 3*s*.) New York: Academic Press Inc.; London: Academic Books Ltd. 1956.

According to the authors of the attractive Introduction to the same subject on modern lines by McLean and Urist (1955), reviewed in these columns a year ago, that slim volume was 'not intended as a full-scale monograph on the physiology of bone'; but we then pointed out that 'the table of contents may well serve as an indication of the scope of such a future work'. We have not had long to wait and now heartily welcome this comprehensive, international volume as an indispensable and authoritative work faithfully reflecting the intensive current interest in bone as a tissue with its biophysical and biochemical problems.

Produced under the general editorship of Dr Geoffrey Bourne, who himself writes on Phosphatase and Vitamin C, it contains 24 chapters, 8 of them in collaboration, covering every aspect of the bone-problem by 28 contributors, 'scattered over Europe, America and the Middle East', who represent all the several disciplines concerned. The editor modestly suggests that the assembly of these contributions 'seemed an intimidating task' and attributes its accomplishment 'on time' to the co-operation of all concerned; but the reviewer cannot refrain from indicating his impression that Dr Bourne himself has done an excellent piece of editorial work on which he is to be congratulated.

Altogether, although the term has been long used in the restricted sense of 'Osteography', this volume—correctly described in the publishers' announcement as 'the first one-volume work to present a critical account of the results of research which could previously be found only in widely scattered periodicals in the field of medical science, biochemistry, biophysics, physiology and anatomy'—constitutes a veritable 'Textbook of Osteology' in the basic sense of the word.

A mere list of the chapter-headings with the names of the contributors would indicate the encyclopaedic scope of the work as a whole and provide a guarantee that the seeker after information in any one of the fields will not be disappointed and in most cases will find a balanced judgement on its outstanding problems. In a composite volume of this magnitude, however, it is natural that the several chapters, though all of interest and individually of special value to particular groups of readers, should vary greatly in the manner and quality of their presentation. They do in fact vary from excellent critical reviews, constituting the bulk of the work and pointing the way to further research, to a few contributions that seem to consist mainly of lists of references repeated in the appended bibliographies. It certainly does not make for easy and attentive reading when, for example, a paragraph consists of two sentences containing over 20 references.

On the other hand, although and probably because the chapters have all been independently written, it is an advantage that they are not necessarily restricted to the actual range of their titles. It is not surprising that individual contributors have found it necessary to relate their reviews of a particular facet of the multiple bone-problem to the subject as a whole, with consequent considerable overlapping from one chapter to another. Indeed, the editor indicates that this overlap to some extent has been planned, and we certainly agree with him that it is an inevitable and most desirable feature. The integration of the whole volume, however, might well have been carried further by cross-references, which are singularly few, and even by a more extensive editorial Preface 'high-lighting' the more salient contributions to certain knowledge and to unsolved problems.

The book gets off to a good start with an excellent chapter by J. J. Pritchard on the 'General Anatomy and Histology of Bone'; and this certainly sets a high standard for the contributions on the morphological aspects of bone which, in spite of the general title, are by no means neglected and indeed constitute about two-fifths of the whole volume. The preface does in fact suggest that the comprehensiveness of this work should make it of particular interest to 'histologists, anatomists, to specialists in orthopedics and pediatrics, and to dentists', and apparently only secondarily to 'biologists, physiologists, biochemists and pathologists'! In addition to the preliminary chapter just mentioned, the morphological aspects are covered by chapters on the Mechanical Problems of Bone, separate chapters on Osteoblast and Osteoclast, and four chapters on various aspects of bone-growth—Osteogenesis, Growth of Bone, Autoradiographic Studies, Repair and Transplantation—with which there is associated an authoritative chapter on 'Skeletal Development in Tissue Culture'. It is in these chapters that most of the overlap occurs and, together with parts of other contributions, e.g. on Ground Substance, Organic Matrix, Collagen Fibres and Ultrastructure, in most of which the technique of electron microscopy and other recent techniques are freely in evidence, they constitute a satisfactory composite picture of bone as a tissue, its histogenesis and growth.

Noteworthy are the tentative conclusions (1) that the osteoblast is an actively secreting cell, that it produces some at least of the materials essential for matrix formation and that 'the evidence is completely against Leriche and Policard's (1926) view that the osteoblast

has no essential role to play in bone formation'; (2) that in the case of the osteoclast 'at the present time the balance of evidence is that osteoclasts bear a causal relation to the absorption of bone, which they effect by superficial or lacunar erosion', although the answer to the important question how they act must remain 'largely speculative until more evidence is forthcoming'; and (3) the conclusive manner in which Dr Honor Fell demonstrates how 'tissue culture has contributed to our knowledge of skeletal structure and physiology in many ways' with the suggestion that 'one of its most important uses in the future may be in the field of hormone and vitamin research'.

But the principal emphasis of the volume, after all, is on the biophysical and biochemical problems presented by bone, since it is in respect of these that most advance is to be expected. Several of the chapters already mentioned e.g. those on Ground Substance and the Organic Matrix, contain chemical sections, and the general chemical problems are fully discussed in separate chapters on Phosphatase and on Calcification (with a subsidiary chapter on the Citrate Content and Metabolism of Bone). Three chapters are devoted to the Vitamins A, C and D, and there are three also on hormones—Steroid Hormones, Anterior Pituitary Regulation (including sections on thyroid deficiency), and the Parathyroids. (In the latter chapter, by the way, the discovery of the parathyroid glands is still attributed to Sandström, and Cave's claim (1953) of priority for Richard Owen (1852) is not mentioned.)

The biophysical aspects of bone are represented in particular by the chapter on 'Ultrastructure and Distribution of Mineral Salts in Bone' and by Dr Janet Vaughan's authoritative chapter on 'The Effects of Radiation on Bone'; and there is a final chapter on 'Pathological Calcification'.

The obvious conclusion from a survey of the scope and content of this work as a whole is certainly, as we have indicated already, that it is an indispensable addition to the library of all who have an interest of any kind in an up-to-date stocktaking of progress in the investigation of the fascinating problems with which it deals. Each chapter is fully documented. Allowing for repetition in the separate chapter lists and for references in the text and tables not there included, rapid calculation indicates that the combined bibliographies extend to some 2500 entries, a very high proportion within the last decade. The illustrations are of a high order; and there are separate, satisfactory author and subject indices.

The only real criticism that may be offered is in relation to the production of the volume. There are certainly not many misprints, none of them disconcerting except perhaps the displacement to the top of p. 138 of a line that should appear at the top of p. 137. But the use of what may seem unnecessarily heavy paper, with a resultant weight of 3½ lb., unfortunately makes the book a little troublesome to handle; and it is a pity also that the publishers have found it necessary to fix the price at such a very high figure.

J. C. BRASH

The Organisation of the Cerebral Cortex. By D. A. Sholl. (Pp. xvi+125 and 12 plates; 18*s*.) London: Methuen and Co. Ltd.

This book contains a critical summary of the principal histological and physiological methods which have been used in the investigation of the cerebral cortex; against this as a background the author develops his view that the organization of the cortex must eventually be described in terms of probability theory and statistical mathematics. It is written quite frankly from a statistical point of view, and to quote the preface is intended partly for readers 'not neurologists or scientists of any kind'. It is doubtful perhaps how far readers with no scientific background will get with a book of this kind; there is no doubt, however, that anyone with a serious interest and at least some knowledge of the problems of organization in nervous tissue, will find it a most useful and very readable work.

Books such as this tend to fall into two classes, which one may call the 'optimistic' and the 'pessimistic'. In the former the author seems to be carried away with an enthusiasm for the new methods he is describing, and may leave the reader with the impression

(usually vague) that at last the key to understanding a complex and puzzling organization has been obtained, and that very little future work and effort will be needed to turn it. The serious student will find such books unsatisfying, though sometimes stimulating. The present work does not come into this class. Though 'pessimistic' is perhaps the wrong word to use, the very critical and cautious attitude adopted by the author to the results from all the methods of investigation he describes, including the statistical, may have a damping effect on those who are hopeful of a quick and easy solution for some of the most intractable of neurological problems. It also gives to the book a conspicuous honesty, shown in the facing of real difficulties and in a reluctance to draw conclusions or make speculations which have inadequate observational justification. The author's interest in, and knowledge of, the histological features of the cortex underlies his whole work, and the excellent series of microphotographs with which it is illustrated is a very pleasant feature of the book. It is satisfactory to find it so fully recognized that whatever mathematical theories or 'models' are developed for the description or explanation of the cortex, they must at least be consistent with the evidence obtained from other methods of investigation.

The early chapters give a good summary of the results obtained from histological examination of the cortex, and of the limitations of such methods. The account of physiological investigations is less satisfactory, mainly because the results are described so shortly as to lose some of their significance. This applies particularly to the account of more recent electrical investigations. The most important chapters are those dealing with quantitative studies, and in particular with methods for the classification of neurones, and for obtaining a measure of their 'connectivity', which have been developed by the author. The concluding chapters on the mode of operation of the cortex and theories of its organization are valuable. They give a useful introduction to the ideas of Lashley, Hebb and others, and a cogent criticism of Ashby and Gray Walter as representatives of the cybernetic school. The discussion of mathematical theories is difficult to follow in detail, but serves its purpose in showing the lines along which the mathematical approach to problems of cortical organization may be pursued. These lines do not as yet appear to have carried us far, and possibly not in the right direction. It does seem, however, that the author has established his point concerning the relevance of mathematical methods. The reader is left with the feeling that they may well be the most appropriate for dealing with the complex interconnected aggregates which form the cerebral cortex, and with a reasonable hope that they will provide a more intelligible and comprehensive explanation of the activities of the cortex than can be given by any of the older methods of investigation. In the present state of our knowledge this is much to be grateful for and as much as can reasonably be expected. This book is a most useful introduction; perhaps it will be followed later by another which will be able to report substantial progress. F. GOLDBY

The Biology of Senescence. By ALEX COMFORT. (Pp. xiii + 257; 41 figures; 25*s.*) London: Routledge and Kegan Paul. 1956.

Progress in medicine over the last hundred years, by improving the chances of surviving the diseases of childhood and early adulthood, has so altered the age distribution of the population of the western world that the proportion of old people is increasing and will increase further. While this large change has been taking place there has, however, been no comparable advance in the specific or maximum age which can be reached by man. The expectation of life for someone who has already reached 65 or 70 years of age is only a little greater than it used to be. An important consequence of the change is a much greater interest in the biological problems of senescence, a subject which had been of little, if any, immediate importance when the chances of survival were, for one reason or another, so slender.

Gerontology, should the subject warrant a separate identity and title, has, indeed, provided ample opportunity for theorizing by ageing philosophers and biologists, from Cicero's *De Senectute* onwards, but the subject has lacked any formulation of its principal problems or general survey of the state of our knowledge about it.

In his recent book, *The Biology of Senescence*, Dr Comfort has sought to gather together all the useful information which could serve to put the problem in perspective and to indicate how present theories succeed or fail to fit the facts. One fact which emerges almost at once is that we are lamentably short of even such basic data as simple life tables of the commonest mammals, and Dr Comfort puts in a strong plea for more effort to be spent in acquiring such information. Having surveyed this field, he goes on to discuss the evidence which bears on theories of ageing. His depressing, but entirely justified, conclusion is that 'all theories of senescence are at present based on unwarrantable assumptions, in the absence of concrete answers to essential questions of fact'. Finally, he considers what seem to him to be the promising lines of approach towards a better understanding of senescence.

The main omission in the book, which the author himself recognizes, is in the social correlates of longevity, though Dr Comfort allows himself to restate the doctor's fundamental belief that the function of medicine is to prolong human life despite any 'neo-Malthusian uproar'. Any discussion of the changes in human performance, either in terms of work or intelligence, with the passing of time, is also left out. This omission is, perhaps, less easy to justify, especially in view of the considerable effort now being directed to the study of the behaviour of older workers in industry.

The only other book with which Dr Comfort's can be compared is the larger *Problems of Ageing* which has reached a third edition in America. That volume is a composite and uneven work with much more bias towards the purely medical aspects of the ageing process in human beings than is found in Dr Comfort's less restricted approach. The two are therefore not strictly comparable, but for anyone beginning to take an interest in the subject there is no doubt that Dr Comfort's book will provide a much better understanding of the general problems and far more stimuli to set him off on the investigation of specific points in the field of gerontology. P. L. KROHN

BOOKS RECEIVED

Human Anatomy and Physiology. By N. D. MILLARD, B. G. KING and M. J. SHOWERS, 4th edition, 1956. (Pp. i–vii, 593, 315 illustrations; 35*s.*) Philadelphia and London, W. B. Saunders Company.

A Shorter Version of The Vertebrate Body. By A. F. ROMER, 1956. (Pp. i–viii, 486, 390 illustrations; 38*s.* 6*d.*) Philadelphia and London, W. B. Saunders Company.

Handbook of Histology. By K. A. STILES, 1956. (Pp. i–ix, 240, 8 illustrations; 22*s.* 6*d.*) New York, Toronto, London, McGraw-Hill.

Correlation between the Function and Structure of Nerve Cells. BY C. VRAA-JENSEN, 1956. (Pp. 88, illustrated; d.Kr. 20.) Copenhagen, Ejnar Munksgaard.

THE GROWTH OF THE HINDLIMB BUD OF *XENOPUS LAEVIS* AND ITS DEPENDENCE UPON THE EPIDERMIS

By PIERRE ANDRÉ TSCHUMI

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I. INTRODUCTION

By studying the fate of carbon particles inserted in chick wing buds, Saunders (1948 *a*) discovered that the wing primordium grows mainly at its distal end and that the future limb segments are laid down in a proximo-distal sequence. Thus, at initial stages, the wing bud consists mainly of mesenchyme which will give rise to proximal segments and the more distal elements are formed successively at the apex of the bud. This apical growth was shown to be dependent upon a thickening of the apical epidermis of the wing bud. This thickening, which extends along the future post-axial and pre-axial borders of the limb and which is found in most vertebrates, is usually called the ectodermal ridge (for other terms see O'Rahilly, Gardner & Gray, 1956). Saunders found that removal of the ridge in the chick completely suppressed the formation of new distal structures, and that only those wing parts which had already been laid down underwent differentiation. His findings have been confirmed and extended by Zwilling (1949, 1955, 1956 *a*, *b*, *c*, *d*) and by Amprino & Camosso (1955).

This work has revived two old problems relating to the experimental embryology of the amphibian limbs. These problems were posed and investigated in the period from 1918 to 1935 (for references see later discussion) without any convincing solution.

The first of these problems is that of the origin and mode of growth of the limb-forming material. The established facts relating to it require brief review.

The mesenchyme cells of the early limb bud originate from a thickening of the somatopleure. In Anuran embryos, the prospective cells of the hindlimb bud leave the coelomic epithelium, migrate to the thickened epidermis opposite and establish a close contact with it (Tschernoff, 1907; Balinsky, 1931; Filatow, 1933; Taylor, 1943). The accumulation of mesoderm cells under the epidermis leads to the formation of a protuberance, the limb bud. The subsequent growth of the bud is apparently due merely to multiplication of its own cells and not to a further supply from neighbouring structures, for, with the exception of nerves and, perhaps, blood vessels, the various histological elements of the limb arise *in situ*. These include the limb musculature, which, as shown by Hamburger (1938, 1939) and Saunders (1948 *b*) in chick embryos and by Byrnes (1898), Lewis (1910) and Detwiler (1934, 1955) in amphibians, is not derived from myotomes. The fact that limb primordia transplanted to various regions of the body develop into complete limbs also strongly supports this view (see Straus & Rawles 1953, pp. 499–503; Hamilton, Boyd & Mossman, 1952, pp. 358–364).

Up to now, little evidence has yet been produced in the Amphibia on the origin of

the prospective material of the various limb segments. Swett (1923), who determined the contribution of the four quadrants of a limb disc to the definitive leg in *Amblystoma*, was not concerned with the growth mechanism itself. The results of his experiments on vital staining were that different parts of the limb are derived from separate quadrants, the largest contribution being made by the dorsal half of the disc. An apical mesodermal growth could have been inferred from an experiment in which vitally stained notochord fragments were placed under the limb ectoderm. They were left behind at the base of the limb as the latter grew out. Unfortunately, Swett abandoned this implantation method which he considered to be unsuitable for his purpose. Later, the xenoplastic transplantations of Schwind (1932) gave results similar to the experiments of Swett. An investigation of the growth of the amphibian limb in the light of Saunders's results is therefore desirable.

The second problem to which Saunders's work has again directed attention is that of an interaction between mesenchyme and epidermis of the limb bud. This is a problem that has in fact received much attention in amphibian experimental embryology.

Numerous experiments in which the role of either the ectodermal or the mesodermal component was tested separately by homo- or heteroplastic transplantation, by rotation or by removal, showed that the factors for localization, polarity, specificity and growth rate of the limbs resided within the mesoderm (Harrison, 1918, 1925, 1931; Detwiler, 1922, 1929, 1933; Suzuki, 1928; Rotmann, 1931). These authors considered the role of the epidermis to be a surbordinate and passive one.

Other observations, however, showed that the epidermis also plays an essential part in the development of a limb: it had already been found by Ekman (1922, cited in Balinsky, 1931) and later by Balinsky (1929, 1931, 1935) and Filatow (1930*a*, *b*, 1932) that the development of early limb mesoderm either could not proceed or was largely inhibited if the mesoderm was separated from its epidermis or if it had been covered by epidermis already determined in other regions. A close association of both mesodermal and ectodermal components seemed to be necessary for the complete development of a limb. Filatow (1928, 1930*b*), who succeeded in causing the development of a limb by transplanting the epidermis alone, was even led to ascribe to the epidermis the leading role in limb development.

Balinsky (1929, 1931, 1935) also held that limb development is the result of a close interaction between epidermis and mesenchyme. But, as the earliest limb mesoderm, though to a very limited extent, is able to differentiate into limb structures without being in contact with epidermis, Balinsky believed that it is the mesoderm which primarily induces the specific epithelial changes. This view is also supported by his observation that in *Triturus* the thickening of the limb epidermis occurs only after contact with the mesoderm has been established, and that undifferentiated head epidermis can take part in limb development if transplanted over prospective limb mesoderm.

Thus, with regard to the earliest phases of limb development, most observations point to the mesoderm as a carrier of the primary limb factors. Yet, it is obvious that the participation of the epidermis in the formation of the limb primordium is of vital importance.

Very little is known about an interaction between mesoderm and epidermis during the later phases of growth and differentiation of the amphibian limb bud. But here again the epidermis seems to play an essential part, for, as in the earlier stages, the mesoderm of larger buds does not develop into full limbs if isolated from the epidermis (Balinsky, 1935) and the destruction of the distal epidermis of a limb bud strongly inhibits the development of the limb (Steiner, 1928). This suggests that in amphibians, as in the chick, the epidermis of the bud is indispensable for its growth. The combination of mesoderm and epidermis of limb primordia from different species (Rotmann, 1933; Heath, 1953) even revealed a specific effect of the epidermis upon the growth rate and the morphology of the limbs, an effect which became apparent only towards metamorphosis.

An attempt has been made to obtain further evidence on the interaction between epidermis and mesoderm in the development of amphibian limbs. In this paper, experiments are reported in which the mode of growth of hindlimb buds of *Xenopus* larvae was investigated with the aid of carmin or carbon particles as markers. A preliminary report has already been published (Tschumi, 1955). These marking experiments revealed that an apical growth mechanism is also present in the limbs of *Xenopus*. This finding led to an investigation of the significance of the apical epidermis.

In the chick, this significance could be determined by mere removal of the ectodermal ridge because this structure does not regenerate. In the Amphibia, however, every part of the epidermis of the limb bud regenerates easily. Thus, special measures were necessary in order to isolate the mesenchyme of the limb bud.

In a first set of experiments, the distal epidermis of limb buds was replaced by skin taken from the head of the larva (Tschumi, 1955). A layer of loose connective tissue which developed between epidermis and mesenchyme soon isolated both components and resulted in an inhibition of further apical growth. Later, clearer results were obtained by removing the epidermis from the whole bud and by transplanting the mesenchyme thus isolated into the abdominal wall of the tadpole. Such buds grew and differentiated without close accociation with epidermis, but they never developed more distal structures beyond those which, according to the marking experiments, had already been laid down. The indispensability of the epidermis for the apical growth of the hindlimb bud of *Xenopus* is thereby demonstrated. Experiments reported below show that the epidermis of the limb bud controls the development of the blood vascular system of the distal regions of the bud.

II. MATERIAL

All experiments were carried out on larvae of *X. laevis* Daud. Breeding was induced by injecting male and female each with 5–600 i.u. of chorionic gonadotrophic hormone.* The larvae were kept in enamelled bowls and fed daily with a suspension of nettle powder. For more details see Gasche (1943), Ochsé (1948), Andres, Bretscher, Lehmann & Roth (1948), Nieuwkoop and Faber (1956).

The experimental animals were reared in an incubator at 20° C., each within a

* Kindly placed at my disposal by Dr Miescher, Ciba, Basel.

drinking glass containing *c.* 200 ml. of water. Changing of water and feeding were carried out daily.

A description of the details of the experimental techniques used will be given at relevant places in the following sections.

III. THE PROSPECTIVE FATE OF LIMB BUDS AT DIFFERENT STAGES

Method

Various regions of the hindlimb buds of *Xenopus* tadpoles were marked by inserting fine carmin or carbon particles into the mesenchyme.

The larvae were narcotized in a solution of MS 222 (Rothlin,* 1932; concentration 1:7000) and held under water in an operation dish by means of two bent glass rods. The bottom of the dish was covered with a soft wax in which the glass rods could be fastened. The animals lay on one side in a groove moulded in the wax. The marking substances were ground and thoroughly mixed with distilled water to yield a smooth paste. A minute amount was then picked up with the point of a very fine steel needle and pricked through the epidermis of the bud into the mesenchyme. One or several marks were made in each bud. Camera lucida drawings of the bud in lateral view were made immediately after marking and again on every second to fourth day until the limbs had reached a length of 2–2·5 mm. The larvae were fixed in 10% formalin when the main skeletal elements of the leg had differentiated (length *c.* 5 mm.). They were stained with methyl green to show the cartilaginous skeleton and cleared in benzylbenzoate. The carmin or carbon particles could then be localized under a dissecting microscope.

The marks tend to fade and to disappear, presumably due to phagocytosis of the particles. In such cases the marks were reinforced by secondary insertions of particles in the same region. Most marks could however be traced during the whole period of observation, which was 1 month or longer.

After 2–3 days the marks, which at first are compact and well delimited, separate into several lumps of variable size. A slight initial spreading is probably caused by a reorganization of the injured mesenchyme area. Very extensive spreading, however, especially along the proximo-distal axis of the limb, was interpreted as a result of growth of the marked area. Stretching was particularly pronounced in marks inserted in distal regions of the limb bud. In order to trace as accurately as possible the distal markings, their most proximally situated particles were used as reference points, for they spread much less and persisted longer than those which came to lie more distally.

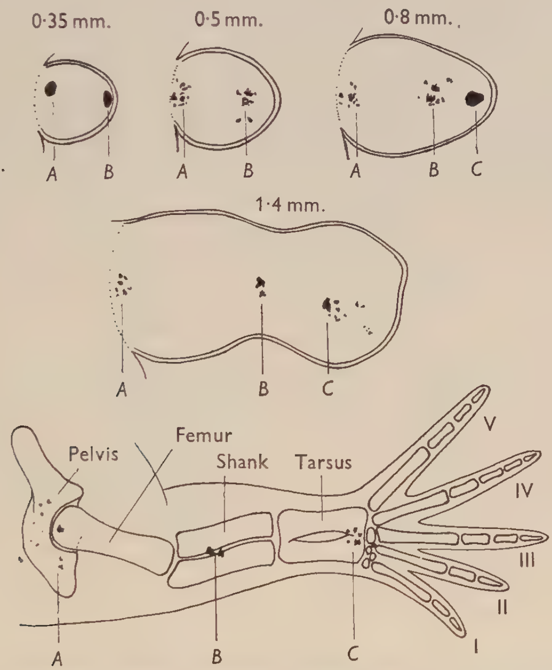
Some 250 marks were inserted into 100 buds which ranged in size from small lens-shaped projections (0·2 mm. length) to limbs in which all the toes were differentiating (*c.* 2 mm. length). Eventually, the ultimate fate of the marks of all the buds were combined into a series of outline drawings by recording the limb regions in which each mark was found at the end of the experiment. A map of the prospective fate of buds at successive stages could thus be established.

* I am indebted to Prof. Rothlin for kindly supplying me with the narcotic.

Results

(*a*) *The growing mesenchyme*

As already stated in a previous paper (Tschumi, 1955), marks inserted in proximal regions of the limb bud spread relatively little. They keep within the base of the limb and at the end of the experiment are found in the pelvic or femoral regions. On the other hand, marks inserted at the tip of the limb bud, immediately under the distal epidermis, rapidly spread. Their most proximal particles are left behind and, depending on the initial stage of the bud, they are finally found at thigh-, shank- or foot-levels (Text-fig. 1).

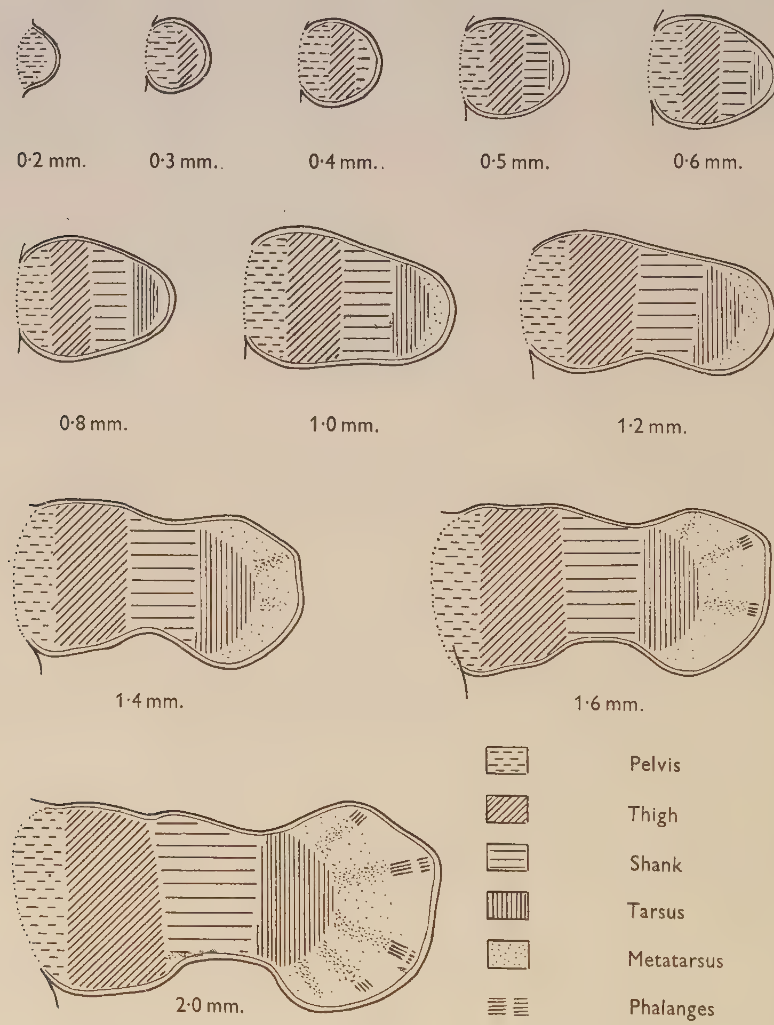


Text-fig. 1. Mark *A* inserted into the proximal mesenchyme of a limb bud comes to lie in the pelvic region. Marks *B* and *C* inserted into the apical mesenchyme at 0·35 and 0·8 mm. bud lengths are left behind and finally lie respectively at shank and distal tarsus levels.

The most likely interpretation of these findings is that the mesenchyme of the limb bud of *Xenopus* grows mainly at its distal end. The map of the prospective fate of various bud regions (Text-fig. 2) shows how the future limb segments are laid down in a proximo-distal sequence at the tip of the bud. This growth mechanism applies not only to the main parts of the whole limb but also to the segments of each toe (Text-fig. 3).

In addition to this apical proliferation, considerable growth also occurs in more proximal areas of the limb bud. This is shown by the fact that the distance between proximal and distal marks or between distal marks and the base of the bud increases (Text-fig. 4, ordinate). Furthermore, marks of intermediate levels eventually spread, particularly along the proximo-distal axis of the bud.

To compare the growth rate of the proximal and distal portions of a bud, the distance between a mark and the tip of the bud and between the same mark and the bud base were measured on the camera lucida drawings. Forty-five marks inserted in buds before the development of the digital plate were used for this purpose. The relative increase in length of each segment was determined for a succession of



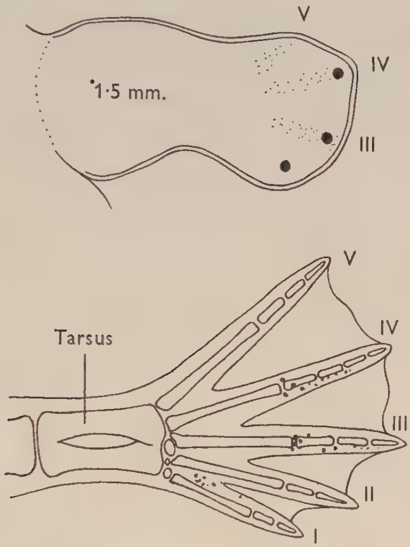
Text-fig. 2. Map of the prospective fate of hindlimb buds ranging in length from 0·2 to 2 mm.

2-day periods and the value for proximal and distal regions of the same bud were plotted against each other diagrammatically (Text-fig. 4). The distribution of the points clearly shows that in a large majority of cases the distal portion of a bud is growing faster than the proximal one.

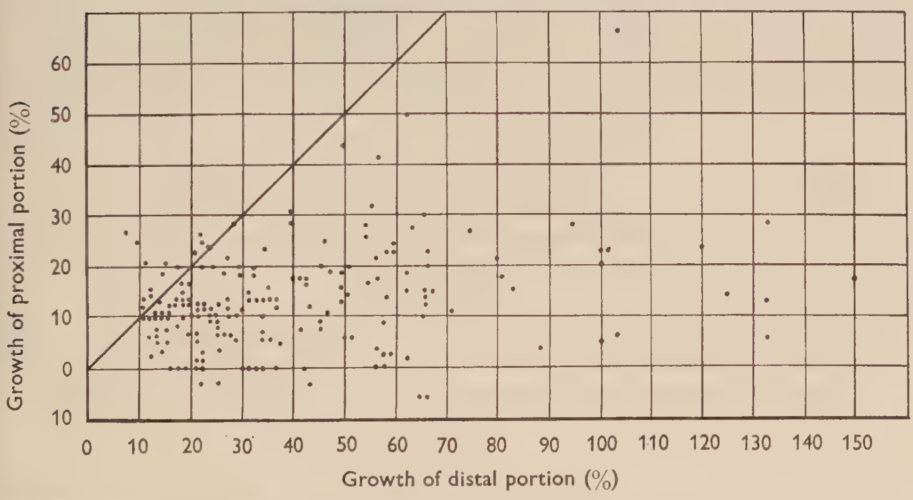
It could be objected that the rapid expansion of distal marks does not necessarily indicate growth but may be due to the particles spreading, for other causes than

growth, more rapidly in the loose apical mesenchyme than in the proximal differentiating tissues. If this were so, however, a high 'growth' rate of distal regions should be correlated with a low 'growth' rate of proximal ones. Text-fig. 4 shows that such a correlation does not exist. It may, therefore, safely be concluded that the expansion, or the lagging behind of marks, is essentially a result of growth.

In short, it may be said that the marking experiments demonstrate that the most intense growth takes place at the apex of the limb bud, where the future limb segments are laid down in a proximo-distal order.



Text-fig. 3. The distribution, in the differentiated toes, of three marks inserted along the margin of the foot primordium.

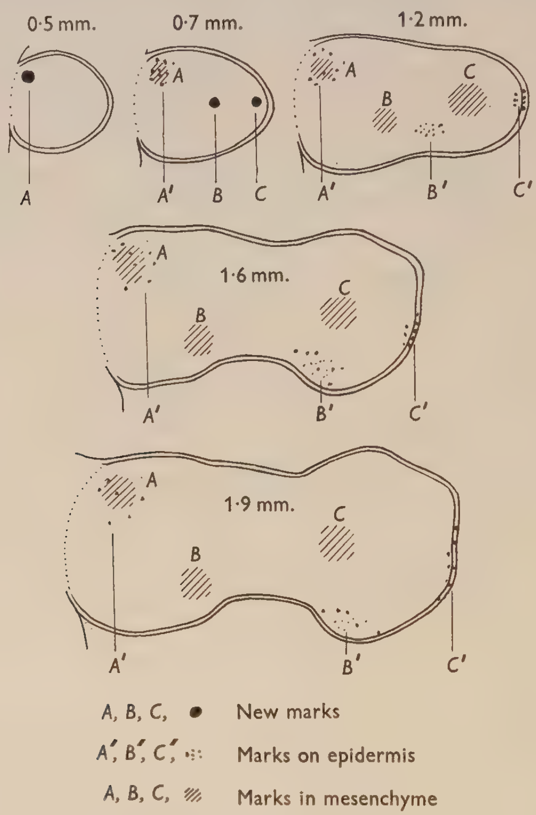


Text-fig. 4. Each dot represents the relative growth of the distal portion of a limb bud plotted against the growth of the corresponding proximal part. Dots above the diagonal line indicate faster growth of the proximal section, dots below the diagonal line faster growth of the distal portion.

(b) The behaviour of the epidermis.

When the carbon or carmin paste is inserted into the mesenchyme, some of the particles frequently remain attached to the epidermis bordering the wound. If, as frequently happens, particles become enclosed within or between epidermal cells, they can be used as indicators for the growth of the epithelium.

In contrast to marks deposited within the mesenchyme, those in the epidermis remain at about the same limb level at which they were inserted (Text-fig. 5).



Text-fig. 5. Marks in the mesenchyme of limb buds are left behind by epidermal marks originally inserted at the same level.

Proximal marks keep at the base of the limb, close to the corresponding mesenchyme mark. Particles inserted in the apical epidermis or along the epidermal ridge remain at the distal margin of the bud, leaving their mesenchymal sister-mark far behind. Finally, marks at intermediate levels stay at these levels or take up a slightly more distal position as the limb grows.

From these observations, therefore, it can be concluded that, except for the very base of the limb, the epidermis continuously glides over the mesenchyme in a distal direction. All epidermal marks spread at nearly equal rate and thus indicate that the epithelium has no particular region of proliferation but grows evenly over all

the surface of the bud. In a paper just received, Hinrichsen (1956) draws similar conclusions from mitotic counts on limb buds of mouse embryos.

The fact that the original apical epidermis maintains its distal position throughout the development of the limb bud may be significant in relation to the special role which the distal epidermis plays in limb development.

IV. THE DEVELOPMENT OF THE ISOLATED MESENCHYME OF LIMB BUDS

The marking experiments described in section III have revealed that new prospective limb material is being laid down at the tip of the limb bud, and later along the distal margin of the digital plate, close under the apical epidermis. Since it has been shown by Saunders in the chick that the ectodermal ridge is essential for the growth of the wing bud, a comparable function of the apical epidermis must be suspected in amphibians.

Experiments already published (Tschumi, 1955) had led to the conclusion that, in *Xenopus*, a close contact between epidermis and mesenchyme was necessary for apical growth. These experiments did, however, not show whether the proliferation of the limb mesenchyme was dependent on a close contact with any kind of epidermis or whether specific limb bud epithelium was required. They also proved unsatisfactory in that 60% of the grafts failed to take. Another method was, therefore, devised.

Very consistent results were obtained by removing the whole epidermis from the limb bud and by transplanting the naked mesenchyme of the bud into the abdominal wall of the same or another tadpole. This wall consists of an epidermis and a pigmented peritoneum with parallel bundles of muscle fibres and some connective tissue lying in between. If the mesenchyme was transplanted between the muscles and the superficial layer (Pl. 1, fig. A), it presumably would develop in contact with the differentiated non-limb epidermis. On the other hand, if transplanted between the peritoneum and the muscles (Pl. 1, fig. B), it would be in isolation from the epidermis. Such sites of implantation have the advantage of allowing observation of the living transplant, including its blood vascular system, during the whole period of the experiment.

Method

The tadpoles were narcotized with MS 222 (1:7000) and transferred to an operation dish filled with a solution of sodium chloride (0.6%) and MS 222 (1:14,000). They were fixed, left side up, with glass rods of appropriate shape in a groove in the wax. The operations were performed with two pairs of very fine and specially ground watch-maker's forceps. No special precautions were taken to prevent infection.

The epidermis was severed at the base of the limb bud, seized with the forceps and carefully pulled away. Particular care was taken to remove as few mesenchyme cells as possible. Slight injuries of the very delicate mesenchyme could generally not be avoided, but too grossly damaged mesenchyme was discarded.

After removing the epidermis from the buds, an opening was made in the skin of the abdominal wall and a space, large enough to contain the transplant, was prepared by introducing the tips of the forceps between epidermis and musculature or between

the latter and the peritoneum. The bud mesenchyme was then gently inserted into the cavity. It is essential that the transplant does not come to lie near the wound, as it may then become exposed and new epidermis may regenerate over it from the abdominal wall (see p. 163).

Seventy-five mesenchymal cores were grafted into the abdominal wall and observed until after metamorphosis. Of those, twenty-nine were lodged between the peritoneum and the musculature. The mesenchyme was taken from limb buds of various stages, the length of which ranged from about 0·3 mm. to over 2·5 mm. (see Text-fig. 2). In twenty-three buds carmin particles had previously been inserted into the tip in order to visualize the growth of this region. As controls, fifteen limb buds with intact epidermis of different stages were transplanted into corresponding situations.

Camera lucida drawings were made of each bud before transplantation and again immediately after the grafting of the whole bud or its mesenchyme. The subsequent growth and development of the grafts were recorded by further sketches. Immediately after metamorphosis the animals were fixed in 10% formalin, then stained with methylgreen to reveal the cartilage and afterwards cleared in a benzylbenzoate-benzene mixture (2:1). In order to render the graft more conspicuous, all the internal organs of the abdomen were removed. The skeleton of each graft was then drawn.

For histological examination, twelve mesenchyme grafts were made and two animals were fixed after 1, 2, 3, 5, 10 and 15 days. Of twenty transplantations of complete buds, two were fixed after ½, 1, 2, 3, 4, 5, 6½, 11 and 16 days. The 8 μ thick sections were stained with Azan.

Results

(*a*) *The development of the controls*

In sections through limb buds transplanted with their epidermis into the abdominal wall no mitotic figures are found within the mesenchyme during the first 2 days following transplantation. Pycnotic cells are common at first, but by the fourth day the pycnoses have disappeared and the transplant has resumed its mitotic activity.

The growth of these buds is nearly as rapid as that of buds in normal position. After the digital plate has developed, however, their shape is becoming atypical due to distortion and bending of the limb. Obviously, the site of transplantation does not permit a straight growth of the limb. Investigation of the grafts after metamorphosis revealed that in all fifteen specimens a complete limb with five toes, three with the usual black claws, had developed. In all these controls the limb is very much distorted owing to bending at the joints, but as a rule, every single skeletal element is straight and of normal shape (Text-fig. 6).

Thus, limb buds transplanted with their epidermis into the abdominal wall always develop into complete limbs, irrespective of the stage of transplantation. This site of implantation, therefore, in no way interferes with the growth and the apical proliferation of the limb bud.

The epidermis of the transplants remains in a healthy condition throughout the period of observation; this is confirmed by the living as well as by the sectioned grafts. In addition, the sections always showed reduplication of the epidermal

sheath round the graft (Pl. 1, fig. C). The secondary inverted epidermis lines the transplantation cavity and is continuous with the epidermis of the bud at its base. It forms, therefore, a closed pocket in which the transplant lies. At some places, bud and pocket epidermis are fused. The sections allow the conclusion that the epidermal pocket arises by a thickening of the bud epidermis, followed by the formation of a basement membrane towards the host tissues and by delamination of the outer cell layers.

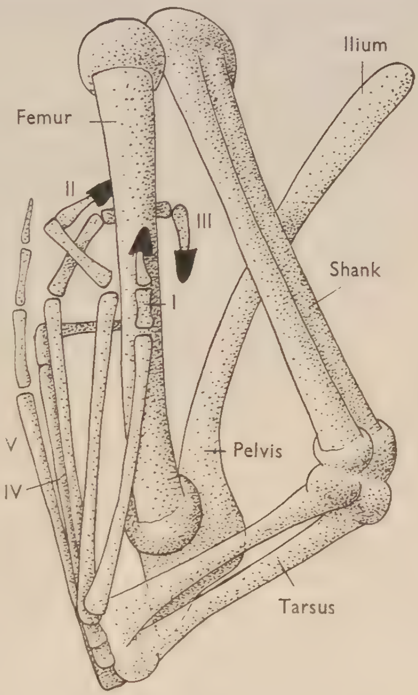
At metamorphosis the grafted limb grows rapidly and frequently protrudes from the body wall. This causes a stretching of the epidermis of the host and of the epidermal layer of the pouch. These layers may eventually burst and leave the transplant, or part of it, freely exposed. Its proper epidermis then becomes continuous with that of the host.

(*b*) *The development of the mesenchyme grafts.*

Two of the seventy-five transplants were resorbed or lost. In six others, the epidermis had presumably not been removed completely as they produced certain of the distal structures that arise from intact buds (see p. 162). The present account is therefore based on the sixty-seven successful grafts.

The grafts heal without much degeneration of cells. One to two days after transplantation, sections through the mesenchyme show a few scattered pycnotic cells, but mitosis is nearly completely absent. After 3 days, most pycnoses have disappeared and by 5 days the mesenchyme has regained its normal appearance with numerous mitotic figures.

Observation of living transplants and examination of sections demonstrated that an epidermis was not regenerated except in the six specimens referred to above. Further, bud mesenchyme transplanted immediately below the belly epidermis does not establish a close contact therewith, as it would with limb bud epidermis. A thin layer of fibrous connective tissue frequently separates most of the graft from the overlying epidermis. After transplantation of mesenchyme between the abdominal musculature and the peritoneum, the muscle layer may partly or completely degenerate. It is then replaced by a thick layer of fibrous connective tissue which keeps the graft separated from the epidermis. (Pl. 1, fig. D). Thus, in both sites of implantation, the mesenchyme develops without a close association with epidermis. Moreover, the only epidermis present in the vicinity of the graft



Text-fig. 6. The skeleton of a limb which developed from a bud grafted with its epidermis into the abdominal wall when only 0·42 mm. long.

differs markedly from limb-bud epidermis by its numerous mucous cells which are fully differentiated.

The grafts became vascularized after about 4 days. From then on they grew steadily but considerably more slowly, however, than did intact buds grafted at the same site. This slow growth can be explained by observations which show that the apical proliferation typical of the growth of normal buds is completely suppressed in the transplants. Thus, the marks inserted into the tips of twenty-three transplants maintained their apical position. The extent to which they sometimes were stretched indicates that the distal region of the mesenchyme was growing at about the same rate as or even less rapidly than the more proximal parts. Again, continued observation of the grafts confirmed that distal structures were in fact no longer being laid down by the isolated mesenchyme. Buds transplanted before the appearance of a digital plate never formed one but slowly developed into elongated tapering structures. In buds grafted shortly after the formation of the plate the latter was maintained but failed to enlarge considerably and to give rise to complete toes.

The effect of the removal of the bud epidermis on the growth of the bud is most strikingly exhibited in animals fixed after metamorphosis and cleared to show the cartilaginous skeleton of the graft. The results obtained are very uniform and can be dealt with summarily.

In most specimens limb-bud mesenchyme grafted between the peritoneum and the musculature, or between the latter and the belly epidermis, differentiated into typical hindlimb parts. Occasionally, distorted cartilaginous structures were found, but they could nearly always be identified as skeletal elements. In addition to the skeletal parts, the grafts also gave rise to other, apparently typically arranged, limb structures, such as connective tissue, muscles, etc. These tissues have not been studied in detail, and will not be considered further.

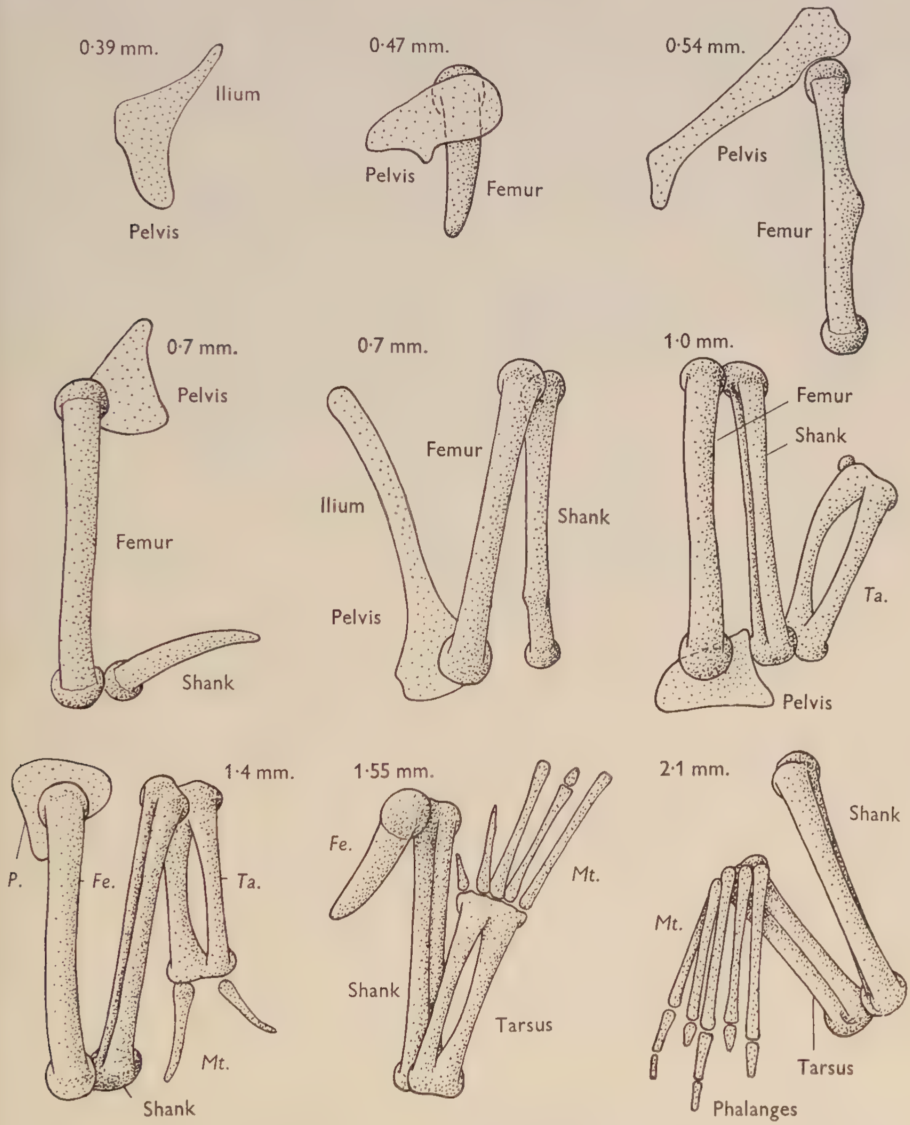
The mesenchyme grafts never developed into complete limbs. Distal elements were always missing to a variable extent, according to the stage of the bud from which the mesenchyme was taken (see Text-fig. 7). Mesenchyme from buds up to 0·45 mm. length only gave rise to cartilaginous elements, which in most cases could easily be identified as parts of the pelvic girdle. Up to 0·55 mm. bud length, the proximal half of a femur or even a complete one developed in addition to the pelvic girdle. The head of the femur was always well developed. In transplants of still larger buds, more and more distal elements were added to the original proximal segments. Thus, complete or proximal parts of the shank appeared in grafts from buds up to 0·9 mm. length, and tarsals and even metatarsals when the buds were up to 1·5 mm. length. Phalanges in increasing number were developed when the length of the donor bud exceeded 1·5 mm.

These findings are summarized on Text-fig. 8. Each vertical line indicates the extent of the differentiated skeletal elements. The dotted curve summarizes the results of the marking experiments and shows which prospective limb parts are laid down at the various bud stages. It can be noted at once that the 'distal' ends of the vertical lines follow the course of the dotted curve. This means that the limb segments which develop from isolated mesenchyme are approximately those which were already laid down at the time of transplantation. Except in cases where the epidermis had not completely been removed, a mesenchymal graft has never been

found to produce more distal structures than those already present as prospective material.

These findings clearly seem to confirm that after the bud epidermis has been removed, the apical proliferation of the limb bud is interrupted and more distal elements are then no longer laid down. Once laid down, however, limb-bud mesenchyme is able to grow and to differentiate without any epidermis at all.

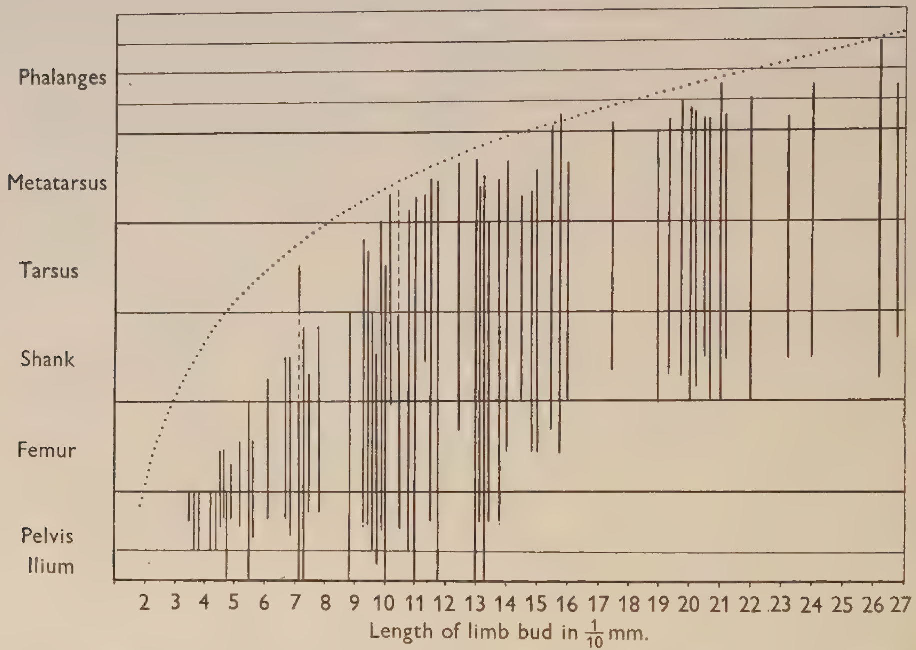
Several transplants, especially from the younger group, developed less distal structures than would be expected from the dotted curve. This may in part be due



Text-fig. 7. The skeletal limb segments which developed from isolated mesenchyme of limb buds which ranged in length from 0·39 to 2·1 mm. *P.* = pelvis; *Fe.* = femur; *Ta.* = tarsus; *Mt.* = metatarsus.

to the loss of distal mesenchyme during the removal of the epidermis. The amount lost will, of course, be relatively greater in very small buds than in larger ones. It may also be that, particularly in younger buds, the most distal mesenchyme is too weakly determined and is not yet self-differentiating.

It will further be seen from the diagram that in most grafts which originate from larger buds, the proximal elements are missing. In order to avoid the implantation of too voluminous a graft, only distal portions of the mesenchyme had been transplanted in these tadpoles. The proximal limit of differentiated structures closely matches the level in the prospective material through which the cut was made (Text-fig. 9). This shows that no regeneration or regulation took place.



Text-fig. 8. The relationship between the limb segments which developed from isolated mesenchyme (vertical lines) and those which, according to the marking experiments, have been laid down in limb buds ranging in length from 0·2 to 2·7 mm. (dotted line).

Isolated limb-bud mesenchyme then develops strictly according to its prospective significance. Its capacity to regulate or regenerate distal and proximal structures is absent or very limited. The wide capability of regulation generally attributed to the limb bud must, therefore, be associated closely with the presence of the epidermis.

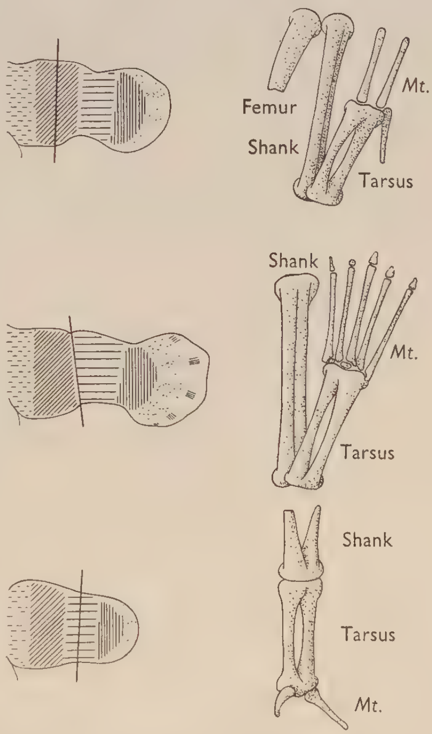
In six out of seventy-five transplants, single or several complete toes did develop. It has already been suggested that in these grafts the epidermis had not completely been removed. Five of these limbs were sectioned after metamorphosis to make sure that an epidermis was present. It was found that all complete toes were in fact covered by epidermis, which in four specimens had differentiated into a black claw at the tip of the toe. In one case, the toe kept well under the epidermis of the host. It was surrounded by two epidermal sheaths (see pp. 158, 159) which were completely separated from the host's epidermis (Pl. 2, fig. E) and which could only have

been derived from epidermis transplanted with the graft. The toes of the four other transplants, however, were free and their epidermis was continuous with that of the host. Most probably these toes originally also had their doubled epidermis, but, as already described (p. 159), the two outer sheaths had presumably degenerated along the toe. This explanation is supported by the fact that in one of the sectioned toes the two outer epidermal layers are still present on one side. In any case, the toes which developed from isolated mesenchyme did so in close association with epidermis presumably transplanted with the mesenchyme.

V. THE DEVELOPMENT OF LIMBS WITH HETEROGENEOUS EPIDERMIS

It can be concluded from the experiments reported above that an apical growth of the limb bud of *Xenopus* does not take place in the absence of the bud epidermis. It can also be inferred from the development of grafts placed closely under the abdominal skin that differentiated epidermis is probably not able to replace the epithelium of the bud. Now, if the limb mesenchyme could resume its apical proliferation after being covered by non-differentiated epithelium originating from any part of the body, it could further be concluded that specific 'limb' properties are not necessarily present *ab initio* in the original limb epithelium.

Ten limb buds, therefore, from which all the epidermis had previously been removed, were transplanted to the abdominal wall or to the head but in such a manner that the distal part of the bud was free (Text-fig. 10). This was achieved by inserting the transplant in the usual way into a pocket prepared under the epidermis or musculature and then by pushing it through an opening made at the other end of the reception cavity. The tip of the mesenchyme transplant was thus freely exposed. It was expected that within a short time the free mesoderm would become covered by epithelial cells originating from the local epidermis bordering the wound.

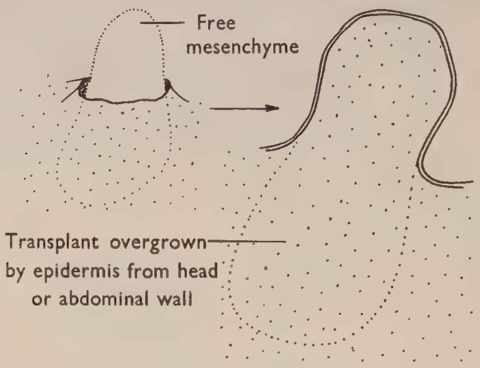


Text-fig. 9. Grafts of isolated mesenchyme of limb buds cut off their base at various levels develop according to their prospective significance.

One of these grafts was lost. Two others withdrew under the local epidermis and developed like isolated mesenchyme. The remaining seven grafts all developed distal structures and toes in a variable number: Specimen (Sp.) 1: five complete toes, but only one toe with claw; Sp. 2: six toes, two of which were duplicates, three with

claws; Sp. 3: four toes, one with claw; Sp. 4: two toes, one with claw; Sps. 5 and 6: each limb with one clawed toe; Sp. 7: one isolated toe with claw.

The high percentage of abnormalities in these seven grafts is most probably not correlated with the site of origin of the epidermis, for similar abnormalities, such as a reduction in the number of toes, duplications and missing claws frequently arise after various experimental interferences with the development of limbs possessing their own epidermis (Tschumi, 1954). The abnormalities seem rather to be due to some inhibition exerted by the tissues surrounding and possibly constricting the



Text-fig. 10. A mesenchyme graft with its tip freely exposed is overgrown by epithelial cells from the site of transplantation and resumes its apical proliferation.

protruding mesenchyme. It is also possible that degeneration of part of the exposed mesenchyme caused its abnormal development. In spite of these considerations, however, the results obtained show that epidermis originating from the abdominal region or from the head is able to promote the apical proliferation of the limb-bud mesenchyme. This supports the view that in *Xenopus* any undifferentiated and indifferent epidermis can be induced to participate in limb development. The primary limb factors can, therefore, be attributed to the mesoderm.

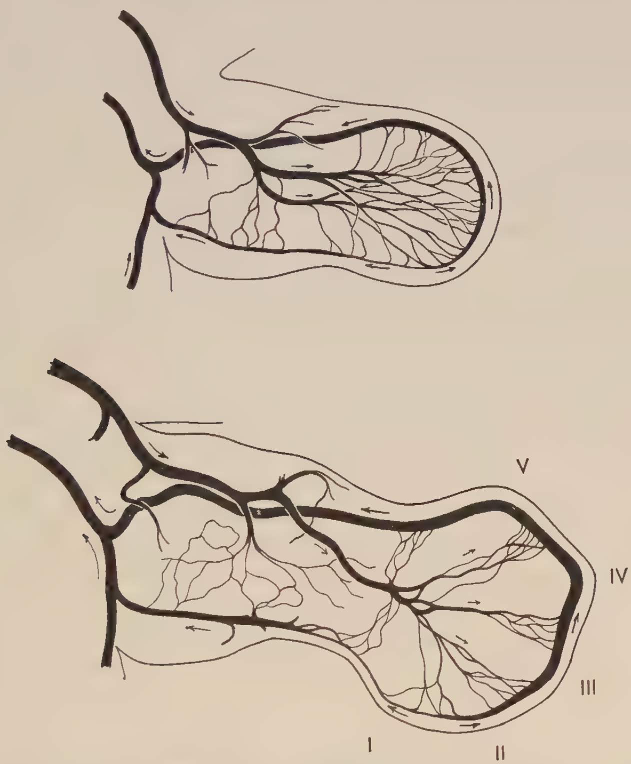
VI. THE RELATIONSHIP BETWEEN THE EPIDERMIS AND THE BLOOD VASCULAR SYSTEM OF THE LIMB BUD

The conclusion that the epidermis is indispensable for the growth of limb buds calls for further investigation of its specific effects upon the developing mesenchyme. There is no evidence that the distal epidermis directly contributes to the growth by giving off cells, as has been postulated for limb regeneration (Rose, 1948). It has been found in this investigation that epidermal cells marked with carmin particles never go into the mesenchyme. There is, moreover, a distinct basement membrane under the most distal epidermis and it would hardly allow a considerable amount of cells to pass into the mesenchyme.

The large number of mitotic figures, especially in the distal part of the bud mesenchyme, suggests that the new proliferating cells arise by cell division within the mesenchyme itself. The source of the required metabolites is presumably the blood, and, as a matter of fact, the actively growing tip of the limb bud is strikingly rich in blood vessels.

Already in the earliest stages, the limb bud of *Xenopus* is vascularized by a plexus of capillaries which reach its tip. When the bud elongates, a large 'marginal' vein develops out of the apical plexus. This vein collects its blood from a system of more or less radially arranged capillaries originating from a central artery (Text-fig. 11). The vein then flows along the distal and post-axial margin of the bud and is a tributary of a large vein at the base of the limb.

The marginal vein develops long before the actual digital plate appears. It becomes larger and more conspicuous when the limb grows, and at the digital plate stage it forms a very large vessel along the plate rim. The marginal vein generally



Text-fig. 11. The main blood vessels in the hindlimb buds of *Xenopus* at two different stages. Note the large marginal vessel and the system of radial capillaries at the tip. Most capillaries of the proximal regions have been omitted.

arises between the primordia of rays I and II and, increasing in size, extends towards the more post-axial toes, bathing, as it were, their proliferating tips. Ray I is related to a smaller vessel flowing in the opposite direction.

The marginal vein closely follows the course of an apical ectodermal ridge of the limb bud (Pl. 2, fig. F). Although in *Xenopus* this ridge is much less conspicuous than in amniotes, it is nevertheless already present at a stage, when the limb bud is only slightly longer than wide. It indicates the future margin of the digital plate.

Observations made during the development of isolated mesenchyme of limb buds showed that a causal relationship probably exists between the bud epidermis (and

possibly the ectodermal ridge itself) and the development of the marginal vessel. The evidence for this conclusion can be summarized as follows:

(1) In the experiment which consisted in transplanting skin from the head over the distal mesenchyme of a bud, it was found that a typical and persistent marginal vein did not develop under the graft. When the mesenchyme was only partly covered by head skin, a marginal vein would develop only along the uncovered region which was proliferating. None could be detected along the inhibited region.

(2) Similarly, a marginal vessel never developed in limb-bud mesenchyme isolated and transplanted into the abdominal wall. All the grafts have carefully been watched for the development of their blood vascular system, but in no case could a marginal vein be seen, not even in limbs with large foot primordia. On the other hand, limb buds transplanted with their epidermis promptly developed a typical large marginal vessel.

(3) In order to get results more reliable than was possible by observing living transplants through the more or less opaque abdominal wall, the blood vascular system of larvae carrying a graft was injected with indian ink. Twenty-one animals which had received a mesenchyme graft 9–12 days previously were used. After narcotizing, a very fine glass pipette filled with dilute indian ink was introduced into the ventricle of the heart and the ink was gently blown into the blood stream. When all the blood vessels looked dark, the animal was fixed in Bouin's fluid and, after washing and dehydrating, cleared in benzylbenzoate.

None of the twenty-one grafts had developed a marginal vein. Their distal portion was only poorly vascularized. Veins occasionally flowing round the tip of the transplant were found to be outside the graft and to belong to the host tissues.

As a control, fourteen animals carrying a graft with epidermis were injected from 6 to 15 days after transplantation. In one specimen, the injection was unsuccessful and no marginal vein could be found. The other thirteen transplants all had a marginal vein which collected its blood from the already mentioned system of radial capillaries.

(4) No marginal vessel can be found in sections through mesenchyme grafted into the abdominal wall (Pl. 2, figs. G, H). Sections of limb buds grafted with their epidermis clearly show the marginal vein (Pl. 1, fig. C).

Thus, it can be concluded that the typical pattern of distal blood vessels, and particularly the marginal vein, does not develop when the apical epidermis of the limb bud is missing.

A developmental relationship between the marginal vein and the epidermal ridge is suggested by recent experiments in which the limb-bud epidermis of *Xenopus* larvae was rotated through 90 degrees. A new marginal vein developed along the rotated ectodermal ridge and, in addition to it, the developing digital plate became orientated like the rotated epidermis (Tschumi, 1956).

A paper on analogous rotation experiments with limb-bud epidermis in the chick has just been published by Zwilling (1956*a*). Zwilling's results are quite comparable to mine as far as the orientation of the mesenchymal limb structures is concerned.

VII. DISCUSSION

The two types of growth of the limb bud and the origin of the prospective limb segments

The marking experiments which have been described indicate that the hindlimb bud of *Xenopus* larvae grows in a way which is quite comparable to that discovered by Saunders in the chick wing, for in both the most actively growing part of the bud is its distal end. The rapid proliferation of the apical mesenchyme results in a continuous laying down of the prospective limb segments in a proximo-distal order. This apical proliferation constitutes the means by which all the prospective limb parts arise. Once laid down, the prospective limb segments go on growing at a rate slightly inferior to that of the distal mesenchyme. The difference between distal and proximal growth rates is statistically significant.

The distinction between 'apical proliferation' and 'growth of material already laid down' is justified, for both types of growth can be dissociated experimentally. It has been shown above that after the mesenchyme of limb buds has been isolated from its epidermis, growth of the material already laid down still goes on. The apical proliferation, however, is completely suppressed. Thus, the latter type of growth requires a close association between mesenchyme and epidermis whilst growth and differentiation of prospective limb tissues already laid down are independent of the epidermis.

Epidermis and mesoderm in the development of the limb bud

This account and the other work to which reference has been made have shown that the participation of the epidermis in limb development is not merely a passive one. Although the present knowledge does not yet permit a definition of the precise roles played by the mesodermal and the epidermal components, one can now nevertheless recognize some of their specific functions. It should, however, be emphasized that the development of a limb is the result of an intricate interaction between the mesoderm and the ectoderm; it is a typical 'kombinative Einheitsleistung' (Lehmann, 1933) and to deal with their roles separately is an inadequate way of approach.

The role which the *epidermis* plays in limb development seems to be confined to promoting the proliferation of the apical mesenchyme and, as stated on page 166, to control its polarity. It thus allows the whole sequence of limb segments to be laid down. The fate of the tissues laid down by this process is obviously no longer determined by the ectoderm, for the mesenchyme transplants grew and differentiated without epidermis according to their prospective significance.

The pattern of blood vessels within the limb bud provides a clue to the nature of the influence of the epidermis upon the mesenchyme. The system of capillaries and the large marginal vessel, which supply the most actively growing region of the bud, do not develop when the mesenchyme is not covered with epidermis. It is tempting to explain the growth-promoting effect of the epidermis by assuming that primarily the epidermis controls the development of the marginal vessel and of the capillary system connected with it. This characteristic pattern of blood vessels may then, in turn, favour, if indeed it does not promote, the multiplication of the apical mesenchyme cells. The absence of apical proliferation after removal of the distal epidermis

could thus be, at least in part, the result of an inadequate vascularization of the distal mesenchyme. The same explanation may also apply to Saunders's inhibition of the apical growth of the chick wing bud after extirpation of the ectodermal ridge.

The idea that an epidermal structure controls the development of the blood vascular system within underlying mesenchyme and that the blood vessels, in turn, should be a determining factor in the growth of a limb is unusual. Yet, Ekman (1913, pp. 139–140) concluded from his experiments that blood vessels were essential for the development and the maintenance of the gills of Anuran larvae, and recently Holtfreter (1955) drew similar conclusions from an experiment in which tail buds of Urodele embryos transplanted to the gill region developed into tails with gill-like projections. Holtfreter suggests that these outgrowths were induced by gill-specific capillaries which invaded the graft.

The ectodermal ridge, which is also present in *Xenopus*, is apparently intimately concerned with the development of the marginal vein. The latter closely follows the course of the ridge in the hindlimb buds of *Xenopus*. A similar relationship has recently been pointed out by O'Rahilly *et al.* (1956) for the limb buds of the human foetus. The experiments mentioned on page 166 indicate that the epidermal ridge does in fact determine the development and the course of the marginal vein. The nature of the relationship between both structures has not yet been ascertained. McAlpine's (1955, 1956) finding that in rat embryos the ectodermal ridge of the limb bud is rich in alkaline phosphatase, and the recent demonstration by Hinrichsen (1956) that the ectodermal ridge of mouse limb buds contains much ribonucleic acid indicate a particular metabolic function of the epidermis. Purely mechanical effects of the epithelium must, however, also be considered. Moreover, the recently discovered polarizing effect of the epidermis upon the mesoderm suggests that the directing influence which the epidermis has upon the marginal vessel could be due to the epidermis primarily controlling the axial arrangement of the distal mesoderm cells. It is hoped that further investigations will help to solve this problem.

It is not likely that the polarizing and other specific 'limb' properties must necessarily (and, in fact, do) reside *ab initio* within the epidermis of the limb primordium, for, as has been shown, isolated limb-bud mesenchyme resumed its apical growth and gave rise to typical and normally polarized distal segments after having been provided with epidermis originating from the head or the trunk region. The polar structure of the epidermis, and the epidermal ridge itself, are thus secondarily induced by the mesoderm. They become, however, indispensable for its further growth and for the establishment of polarity within the newly arising structures. For realizing its own inherent potencies, the mesoderm of the limb primordium thus uses, as it were, the epidermis for a templet, which it first endows with certain properties and which, in turn, reacts upon its originator.

The only requirements for the epidermis, apparently, are that it be originally indifferent and non-differentiated and in close contact with the mesenchyme. It was seen that differentiated epidermis from the head or the abdominal wall does not promote the apical proliferation of mesenchyme. Balinsky (1931) and Filatow (1930*a*, 1932) were able to inhibit limb development by transplanting determined head or gill epidermis over prospective limb mesoderm. Zwilling (1955) showed that the same is true also in the chick. Mesoderm of limb buds covered with epidermis from tail,

neck or back developed like ectoderm-free mesoderm, i.e. it gave rise only to girdle elements occasionally associated with parts of the stylopodium.

The epidermis, moreover, is not concerned with the determination of the fore- and hindlimb properties. In Zwilling's experiments, combinations of wing mesoderm with leg ectoderm and the reverse always resulted in limbs developing according to the mesodermal origin. Similarly, five hindlimb buds of *Xenopus*, on which I transplanted epidermis of the forelimbs, developed into hindlimbs.

A leading role of the mesoderm in determining typical fore- or hindlimb structures, particularly of ectodermal origin, has been found by Saunders (1951), Cairns & Saunders (1954), Cairns (1955) and Saunders, Gasseling & Cairns (1955) in the chick. After transplantation of leg mesoderm under wing ectoderm, feathers of the leg type, toes, scales and claws developed on the wing. A thin layer of mesoderm immediately underlying the ectoderm had the same inducing effect as mesoderm taken from deeper regions.

These contributions, and the work reported here, would seem to confirm the view of Harrison that the specific limb-determining factors originally reside within the mesoderm and that the latter induces the epidermis to participate in limb development. This participation, however, is a very active and indispensable one indeed.

Mosaic development, regulation and regeneration in limb buds

A further point of interest in the present investigation is that isolated mesenchyme always develops according to its prospective significance. In the first place, this finding strongly confirms the results of the marking experiments. Secondly, it raises the question of whether the development of a limb bud is not rather of the mosaic than of the regulation type.

It is generally accepted that in amphibians the primordium of the free extremity is a highly regulative, equipotential system (Harrison, 1918; Mangold, 1929). Yet, no significant regulation is exhibited by limb mesenchyme developing in the absence of epidermis, neither at the distal nor at the proximal end. One wonders if several instances of 'regulation' are not, in fact, the result of a regeneration of missing mesenchyme areas by the proliferating epidermis-mesenchyme system.

Like growth in normal development, regeneration of larval or adult limbs requires the presence of an undifferentiated epidermis being directly in contact with the mesodermal tissues. No regeneration occurs after isolation of epidermis-free amputated limbs under trunk skin (Polezajew & Faworina, 1935), after transplantation of differentiated skin to an amputation surface (Milojevic, 1924; Tschumi, unpublished), after rapid wound closure by skin with dermis, or after the development of a layer of connective tissue between stump tissues and epithelium (Schotté & Harland, 1943 *a*, *b*; Thornton, 1949, 1954). On the other hand, regeneration can be induced in normally non-regenerating forms by transplanting larval epidermis to the amputated limb or by allowing the amputation surface to be covered by thin dermis-free epithelium (Gidge & Rose, 1944).

Thus, the ability for extensive repair exhibited by the whole limb primordium bears a strong resemblance to typical regeneration in so far as both are dependent upon the presence of an adequate epidermis. The regenerative processes with which

the epidermis is specifically concerned mainly involve proliferation of mesenchyme, though the existing tissues probably also undergo some degree of reorganization. Suppression of the possibility of regeneration by removal of the epidermis clearly shows how little regulation is possible with mesenchyme alone.

It can be concluded that the development of the mesenchyme of limb buds has much in common with mosaic development. There is, therefore, no real discrepancy between the mosaic development of the girdle (see Nicholas, 1955) and the supposed regulative development of the free appendage. Mesenchyme which has been laid down by the proliferating epidermis-mesoderm system behaves like a mosaic when isolated from the distal epidermis. This must particularly be evident for the girdle elements as they are the first ones to be laid down and to loose their contact with the apical epidermis.

VIII. SUMMARY

1. Carmin or carbon particles inserted into the mesenchyme of hindlimb buds of *Xenopus* larvae revealed that the tip of the bud grows more rapidly than do the more proximal areas. This apical region of proliferation lays down the prospective limb segments in a proximo-distal sequence. A map of the prospective significance of limb buds at various stages has been established.

2. Particles which come to lie within the epidermis of limb buds show that there is no apical proliferation of the epidermis. The latter grows uniformly over all the surface of the bud and leaves behind the mesenchyme which has been laid down at the tip of the primordium.

3. The mesenchyme of limb buds, from which the epidermis has been stripped away, was transplanted into the abdominal wall. Here it developed in isolation from epidermis and gave rise only to those proximal limb segments which had already been laid down at the time of transplantation. Thus, the proliferation of the apical mesenchyme is suppressed when the epidermis of the limb bud is missing. Growth and differentiation of mesenchyme already laid down are, however, independent of the epidermis.

4. Isolated mesenchyme of limb buds overgrown by epithelial cells originating from the head or the abdominal wall resumes its apical proliferation. Specific limb properties do not, therefore, reside *ab initio* in the epidermis, but the latter must be indifferent, undifferentiated and in close contact with the mesenchyme.

5. The large apical marginal blood vessel of limb buds and the system of radial capillaries associated with it do not develop in isolated mesenchyme. It is concluded that the epidermis controls their development and, therewith, the blood supply required by the actively proliferating apical mesenchyme.

6. The ectodermal ridge of the limb of *Xenopus* controls the course of the marginal vessel and the polarity of the proliferating mesodermal tissues.

7. Isolated mesenchyme develops according to its prospective significance with only little regulation. The high capability of 'regulation' attributed to the limb buds of the Amphibia might thus be regarded as regeneration, as both similarly depend upon a contact with non-differentiated epidermis.

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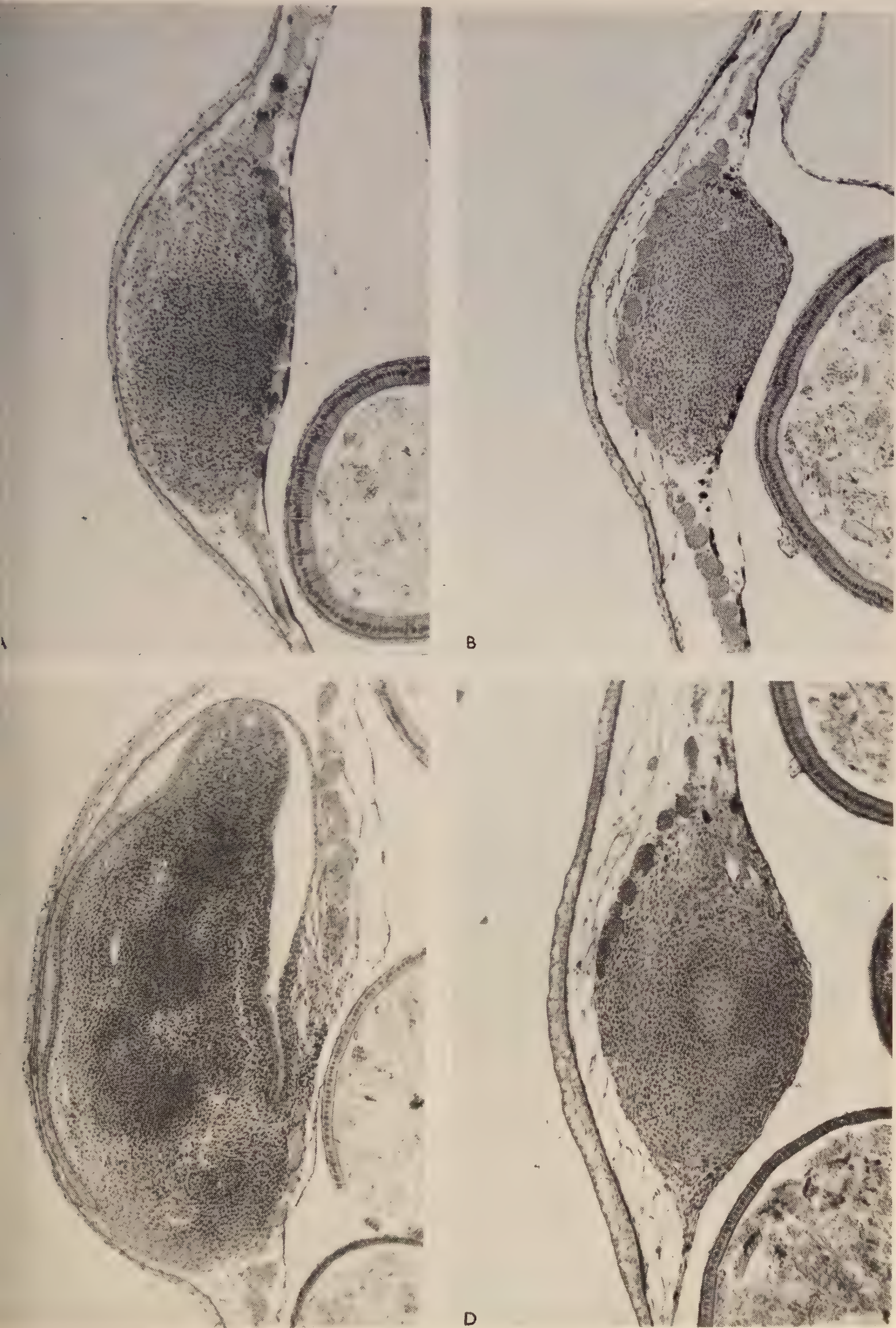
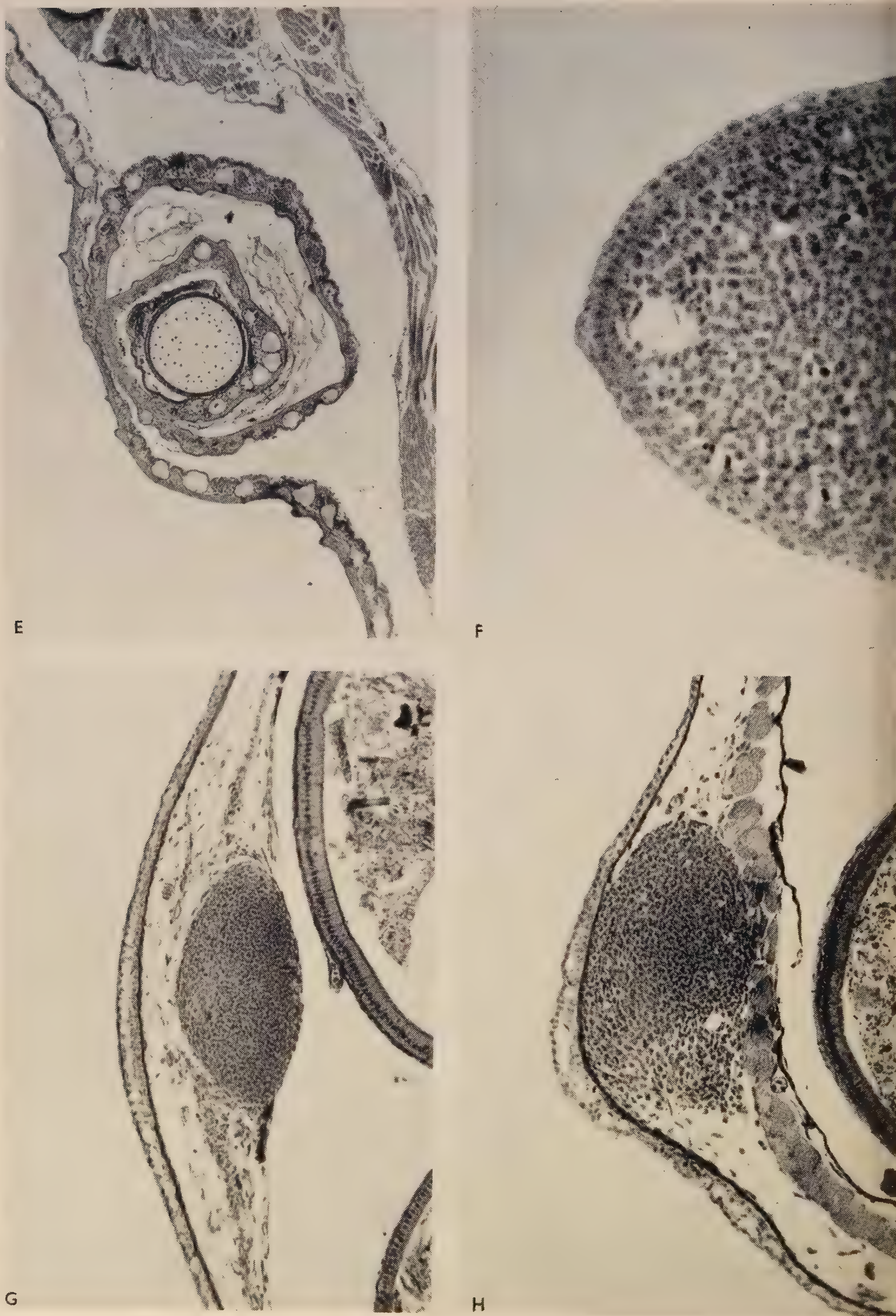


TSCHUMI—Growth of the hindlimb bud of *Xenopus laevis*

(*Facing p.* 172)



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EXPLANATION OF PLATES

PLATE 1

Fig. A. The mesenchyme of a limb bud grafted 1 day previously between epidermis and musculature of the abdominal wall.

Fig. B. The mesenchyme of a limb bud grafted 3 days previously between musculature and peritoneum of the abdominal wall.

Fig. C. Section through a limb bud grafted with its epidermis into the abdominal wall, showing duplication of epidermis and apical marginal vessel; 8 days after transplantation.

Fig. D. Mesenchyme graft after partial degeneration of the abdominal muscles. A thick layer of connective tissue separates the graft from the epidermis of the host.

PLATE 2

Fig. E. Section through a toe which developed within the abdominal wall of a host from a mesenchyme graft. It is surrounded by two layers of epidermis presumably derived from epidermis transplanted with the mesenchyme.

Fig. F. Section through the tip of a hindlimb bud of *Xenopus*. Note marginal vessel and apical ectodermal ridge.

Figs. G & H. Sections through the distal ends of mesenchyme grafts. The tip of the graft has no close contact with the abdominal epidermis and no apical marginal vessel can be seen.

THE ACTIVITY OF THE TRIGEMINAL PLACODE IN THE SHEEP EMBRYO

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INTRODUCTION

It is widely accepted in the literature that the trigeminal ganglion differentiates principally from the neural crest in all the vertebrate classes. In fish and amphibian embryos the neural crest rudiment of the ganglion is usually augmented by a moderate contribution of cells which detach in a mass from one or several placodal thickenings of the surface ectoderm. There is abundant evidence that the neuroblasts of the trigeminal ganglion may differentiate from both these sources. Knouff (1927) has summarized the details of the pattern of development of the trigeminal nerve, particularly in the lower forms, and Ariëns Kappers (1941) has reviewed the extensive literature on the problem of placodal activity in the vertebrates.

The question of the participation of placodal cells in the establishment of the trigeminal ganglion among mammals has attracted little attention and many of the available descriptions are incomplete owing to the difficulty of collecting sufficient material and the impracticability of experimental verification. As early as 1885 Froriep noted the intimate fusion of thickened epibranchial ectoderm with the ganglia of nerves VII, IX and X in bovine embryos, but failed to find any similar sites in contact with the ganglion of nerve V. Chiarugi (1897) described the intervention of ectodermal cells in the formation of the trigeminal ganglion in the guinea-pig, while in the same species Da Costa (1931) finally reached a negative conclusion after re-examining his earlier results (1923, 1925). The reality of the process of placodal budding in the squirrel has been accepted by Völker (1922), but denied by Weigner (1901), who considered that the ganglion developed exclusively from the neural crest. Further denials of placodal participation have been expressed in the rat by Adelmann (1925), in the cat, roe-deer and man by Holmdahl (1928) and in man by Weigner (1901). In contrast to these views a definite ectodermal contribution to the developing trigeminal ganglion in the human embryo has been claimed by Atwell (1930), Bartelmez (1924), Bartelmez & Evans (1926), Campenhout (1948), Giglio-Tos (1902) and Wen (1928). The investigations of Campenhout (1935, 1936, 1937) in the pig, Coërs (1946) in the rabbit and Halley (1955) in the cat have all shown a migration of placodal cells from a series of small thickenings scattered within the broad 'area V' of the lateral head ectoderm.

The only reference to the sheep was made by Froriep (1885), who found no evidence of placodal sites over the trigeminal ganglion in a small number of embryos. In the present study, which provides a more detailed account of the behaviour of the trigeminal placode in this species, an attempt has been made to visualize the pattern and extent of placodal activity in space and in time by recording the presence of migration points and by counting their cells in each embryo examined.

MATERIALS AND METHODS

This report is based on the examination of fifty-three sheep embryos ranging in size from 14-somites to 9·5 mm. crown-rump length (15–23 days). Many of the younger specimens are the property of Prof. J. D. Boyd, who kindly allowed me to examine his collection of sheep embryos of known age; all these embryos were cut serially at 6μ and stained with Heidenhain's haematoxylin alone or with Weigert's haematoxylin and orange G. The remaining material was collected in the Bristol abattoirs and was fixed in Bouin's fluid, dehydrated and stored temporarily in 2% celloidin before infiltration with paraffin wax. Serial sections were then cut at 7 or 10μ and stained with Ehrlich's haematoxylin and eosin Y.

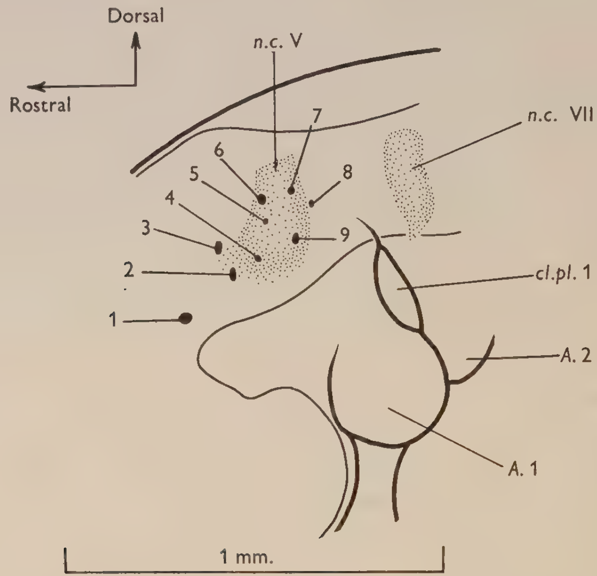
Profile reconstructions of the head region were prepared by the graphic method, using selected embryos which were cut serially at 10μ. Alternate sections were drawn at a magnification of fifty diameters using an 'Edinger' projector and the detail was transferred to an enlarged outline traced on millimetre squared graph paper. This method will reconstruct fine detail such as the pattern of placodal contacts with an acceptable degree of accuracy. Text-figures 1–3 are tracings of the trigeminal nerve taken from reconstructions of the entire head region prepared by the graphic method.

OBSERVATIONS

14–30-*Somite stage*, 15–19 *days*

The trigeminal neural crest is already present in the 15-day embryo in which the neural tube is still open at the mid- and hind-brain levels. As in the rat (Adelmann, 1925) it is quite independent of the facial crest, from which it is separated by a distinct gap. At first the trigeminal crest is an extremely diffuse mass of cells lying beneath the low cuboidal epithelium of the surface ectoderm. Later, in the 23-somite embryo of 18 days, the crest cells become grouped in a more compact mass except along the distal edge where they come into intimate contact with the epidermis. By the 26-somite stage the trigeminal crest has grown into an oblong mass which presents a well-defined outline at only two of its borders: dorsally, where the cells lie in close apposition to the hind-brain and caudally, where they are clearly delimited from the mesenchyme (Text-fig. 1). Rostrally the margin of the crest rudiment is indefinite since the cells are more loosely arranged and appear to be growing forwards. Distally the crest is apparently continuous with the mesenchyme of the mandibular arch and here, too, a distinct margin is lacking. The greater part of the lateral surface of the trigeminal crest now lies close beneath and almost in contact with the epidermis; at isolated points this bears minute cellular thickenings which produce local irregularities of the deep surface of the epithelium. These placodal thickenings are distributed not only within the present limits of the crest, but also ahead of it in an area below which the rostral tip will shortly grow to form the anlagen of the ophthalmic lobe of the ganglion. The placodal sites are produced by local mitotic proliferation and appear in section either as low cushion-shaped thickenings or as short free ending spurs which project obliquely downwards towards the crest. In the 26-somite embryo which was reconstructed nine placodal sites occur on the left side (Text-fig. 1) and seven on the right. On either side four of

these sites are connecting spurs which project down to fuse with the crest (Table 4). The total number of cells in all the placodal thickenings of this embryo is sixty-five on the left and fifty-seven on the right side; further details of the shape and size of individual thickenings are summarized in Table 1. In the present series of embryos the 23-somite stage marks the onset of placodal activity, since with only one exception placodal thickenings are present regularly on both sides of six embryos of this stage (Table 4).



Text-fig. 1. Profile reconstruction of the left trigeminal nerve in a 26-somite sheep embryo (no. 10). Magnification ×50. The placodal sites are numbered 1–9 and brief details of their shape and number of cells are given in Table 1.

Table 1

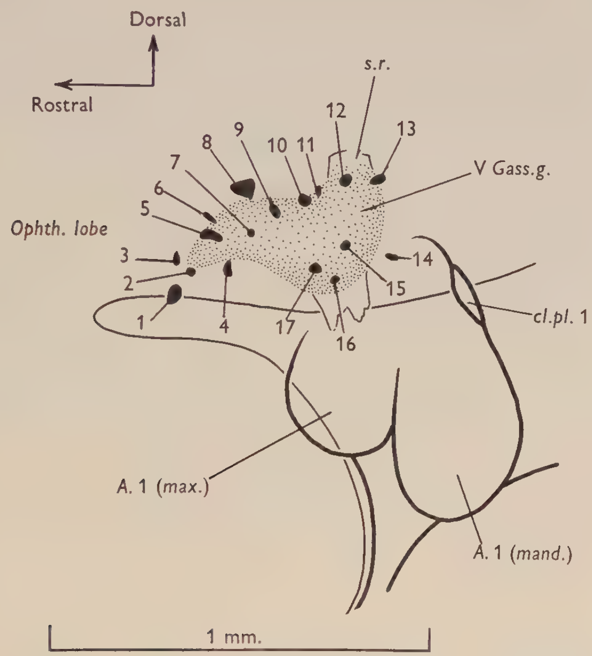
Site	
1	10-cell spur
2	7-cell spur
3	7-cell cushion
4	9-cell cushion in contact with the neural crest
5	6-cell cushion in contact with the neural crest
6	8-cell cushion
7	6-cell cushion in contact with the neural crest
8	6-cell cushion in contact with the neural crest
9	6-cell spur
Total	65 placodal cells

31–35-*Somite stage*, 5·5–7·0 *mm.* (*crown-rump length*) *embryos*, 20–21 *days*

In the 6 mm. (32-somite) embryo the neural crest cells have condensed into a more compact mass of young preneuroblasts so that the external shape of the ganglion is more precise than in the earlier stages (Text-fig. 2). A flat band of fine nerve fibres growing out from the dorsal edge of the caudal part of the ganglion marks the appearance of the dorsal sensory root. From the rounded ventral border a short outgrowth of fibres foreshadows the development of the maxillary and

mandibular rami. The most striking advance, however, is the development of the tapering ophthalmic lobe in which the present compact arrangement of preneuroblasts contrasts with the diffuse character of this portion in the earlier embryos. Externally a low maxillary bud forms a new feature alongside the more prominent and lengthened mandibular process.

During the 6 mm. stage the proliferation of placodal cells by the epidermis reaches the climax of activity (Table 4). A reconstruction of a 6 mm. embryo shows



Text-fig. 2. Profile reconstruction of the left trigeminal nerve in a 32-somite sheep embryo (no. 13). Magnification × 50. The placodal sites are numbered 1–17 and brief details of their shape and number of cells are given in Table 2.

Table 2

Site	
1	10-cell cushion
2	5-cell cushion
3	7-cell cushion
4	9-cell file
5	10-cell cushion
6	5-cell file connecting with the ganglion
7	6-cell cushion
8	15-cell cushion connecting with the ganglion
9	7-cell spur
10	8-cell cushion
11	5-cell file
12	8-cell spur
13	8-cell cushion connecting with the ganglion
14	5-cell file
15	7-cell spur connecting with the ganglion
16	5-cell cushion
17	8-cell spur
Total	128 placodal cells

seventeen placodal sites distributed over the ganglion and ahead of the ophthalmic lobe (Text-fig. 2). Some of these sites are low cushions, others are short spurs and the remainder are narrow files of cells projecting towards the outer face of the ganglion (Table 2). Four of these sites consist of streams of epidermal cells connected with the ganglion and may be interpreted as placodal cells fixed during the course of their detachment, migration and incorporation into the ganglion. The total number of cells counted in all the thickenings in this embryo reached 128 for the left and 123 for the right side; in another embryo of 6·8 mm. crown-rump length 114 and 116 cells were counted (Table 4).

The proximity of the trigeminal neural crest to the thin ectoderm in most early embryos makes the placodal thickenings difficult to identify without a careful scrutiny. In a favourable section (Pl. 1, fig. 1) from a 33-somite embryo three placodal sites are easily recognisable with the $\times 10$ objective. Both the upper and the lower spurs consist of streams of twelve cells which have been fixed during the course of their migration toward the ganglion. The middle site is a flat cushion-shaped plaque which is not yet connected to the ganglion. In the extreme rostral part of the placodal field the epidermal thickenings are more easily seen, since the ophthalmic tip of the ganglion has not yet grown forward to this level (Pl. 1, figs. 2, 3). The upper site in Pl. 1, fig. 2, is a typical cushion-shaped thickening which in this section contains eight nuclei out of a total of ten cells.

The lower conical site shows three nuclei and a single mitotic figure out of a total of five cells. On the right side of the same embryo (Pl. 1, fig. 3) the upper site contains five nuclei out of a total of ten cells for the whole spur. A pinched attenuated nucleus present at the base of this spur probably represents an early stage in the detachment of placodal cells. In the smaller lower site four nuclei are visible, but the whole thickening contains six cells.

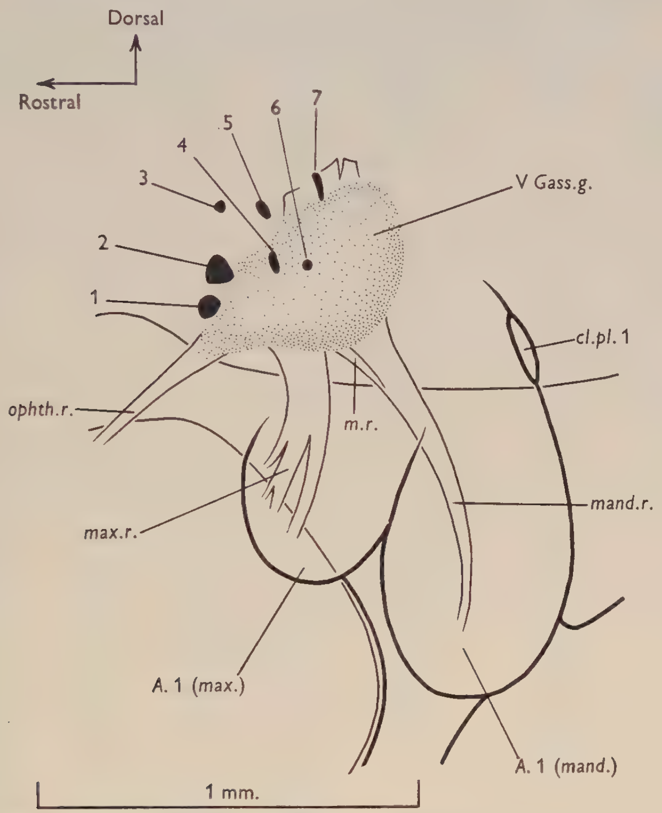
8·0–9·5 *mm.* (*crown-rump length*) *embryos*, 22–23 *days*

By the 8 mm. (42-somite) stage the definitive pattern of the trigeminal nerve has been established (Text-fig. 3). The ganglion possesses a short, but broad, sensory root in which separate fascicles are not apparent until the 10 mm. stage. Although it is larger and more rounded the present shape of the Gasserian ganglion reflects the triangular form seen in the previous stage; the rostral angle becomes clearly identified as the ophthalmic lobe for it now sends forward a fibrous ophthalmic ramus in the direction of the eye. The maxillary ramus penetrates deep into the maxillary bud before terminating in a spray of fine nerve bundles. Slightly caudal to this the broad sensory root of the mandibular ramus emerges from the ganglion and after a short independent course receives a slender motor root; beyond this union the mandibular ramus passes down the axis of the arch as a mixed nerve.

Placodal activity continues in the 8 mm. embryos although at a reduced pace, which is reflected in the smaller number of spurs present and in a lower total of placodal cells. Of the seven sites present on the left side of the reconstructed 8 mm. embryo two connect with the ganglion and the total number of cells is eighty-six (Table 3). The right side of the same embryo bears nine sites with a total of eighty-six placodal cells (Table 4).

In all embryos of this stage the field of placodal activity is characteristically

displaced over the rostral part of the ganglion and even minute thickenings over the maxillo-mandibular part are infrequently found. As the epidermis becomes lifted away from the surface of the ganglion by an increasing thickness of mesenchyme the placodal sites become more conspicuous than in the earlier embryos.



Text-fig. 3. Profile reconstruction of the left trigeminal nerve in an 8 mm. (crown-rump length) sheep embryo (no. 15). Magnification ×50. The placodal sites are numbered 1–7 and brief details of their shape and number of cells are given in Table 3.

Table 3

Site	
1	25-cell spur
2	25-cell spur connecting with the ganglion
3	6-cell cushion
4	8-cell file connecting with the ganglion
5	7-cell cushion
6	7-cell spur
7	8-cell file (Pl. 1, fig. 4)
Total	86 placodal cells

Some placodal sites appear as narrow files of cells (Pl. 1, fig. 4) which are about to detach from the epidermis and migrate to the ganglion, while others are of the minute conical type. A further type of placodal thickening, which is frequently present over the ophthalmic lobe of the trigeminal ganglion during this stage, is

Table 4. *The incidence of placodal thickenings in relation to the trigeminal ganglion in 50 sheep embryos. The number of placodal sites which make connexion with the ganglion is given in brackets. The letter 'C' preceding the serial number distinguishes embryos of Prof. Boyd's collection.*

Embryo no.	Age (days)	Crown-rump length (mm.)	Somite count	No. of placodal sites		Total no. of placodal cells		
				Left	Right	Left	Right	
C 16	16	—	14 s.	—	—	—	—	
C IV	18	—	23 s.	1 (1)	—	5	—	
C 10	18	—	23 s.	6 (1)	3 (–)	42	24	
C 4	18	—	—	4 (1)	8 (1)	44	69	
C 24	18	—	—	5 (–)	9 (2)	30	58	
C 6	19	—	—	6 (2)	7 (1)	33	54	
C 7	19	—	—	2 (1)	5 (1)	19	22	
10	—	—	26 s.	9 (4)	7 (2)	65	57	(Text-fig. 1)
*C 1	21	—	—	4 (–)	5 (–)	30	27	
*C 2	21	—	—	2 (–)	1 (–)	15	4	
C 23	19	—	—	8 (–)	7 (–)	60	35	
40	—	—	30 s.	10 (2)	9 (4)	57	71	
C 25	20	—	31 s.	5 (1)	3 (–)	29	21	
C 26	20	—	—	4 (1)	2 (1)	20	13	
C 27	20	—	—	6 (1)	4 (2)	32	20	
C 29	20	—	—	—	6 (–)	—	42	
C 11	20½	—	—	2 (1)	3 (–)	15	25	
C 12	20½	—	—	7 (3)	8 (3)	53	65	
28	—	5·5	—	7 (2)	5 (–)	61	31	
12	—	5·8	32 s.	10 (2)	7 (3)	64	61	
13	—	6·0	32 s.	17 (4)	11 (4)	128	123	(Text-fig. 2)
11A	—	6·0	33 s.	10 (4)	8 (–)	86	46	
11B	—	6·0	33 s.	10 (4)	7 (3)	59	45	
42	—	6·5	—	5 (–)	3 (1)	21	15	
14	—	6·8	35 s.	13 (5)	11 (4)	114	116	
36	—	7·2	—	6 (1)	5 (2)	34	33	
C 8	22	8·0	—	3 (1)	2 (1)	17	19	
15	—	8·0	42 s.	7 (2)	9 (3)	86	86	(Text-fig. 3)
4	—	8·0	—	7 (2)	5 (1)	47	34	
561	—	8·0	—	6 (3)	5 (3)	60	84	
16	—	8·2	—	6 (1)	5 (–)	54	41	
17	—	8·2	—	9 (4)	8 (2)	61	62	
C 9	22	8·5	—	4 (1)	1 (–)	25	5	
18	—	8·5	—	6 (1)	4 (2)	47	37	
19	—	8·5	—	3 (2)	3 (1)	53	26	
C 13	22	8·7	—	6 (1)	5 (2)	72	93	
C 14	22	9·0	—	7 (–)	6 (3)	60	75	
C 3	22	9·0	—	3 (2)	3 (1)	43	17	
20	—	9·0	—	5 (2)	4 (–)	69	31	
25	—	9·0	—	5 (–)	2 (–)	34	21	
21	—	9·0	—	2 (–)	2 (–)	12	10	
537	—	9·0	—	—	2 (–)	—	17	
551	—	9·0	—	1 (1)	1 (–)	10	5	
552	—	9·0	—	3 (–)	—	22	—	
8	—	9·0	—	2 (–)	—	11	—	
C 20	23	9·2	—	—	1 (–)	—	5	
C 21	23	9·2	—	—	—	—	—	
550	—	9·2	—	—	—	—	—	
23	—	9·5	—	1 (1)	1 (1)	6	28	
22	—	9·5	—	—	—	—	—	

* C 1 and C 2 are twins, but have the developmental structure of a 19-day embryo.

illustrated in Pl. 1, fig. 5. It is similar to sites 1 and 2, Text-fig. 3, and consists of a massive stream of from twenty-five to thirty cells. Similar massive spurs of up to thirty-six cells are present in the ectoderm over the ophthalmic lobe in several embryos of between 8·0 and 9·2 mm. in length (Table 5). The majority of these spurs are connecting streams similar to that shown in Pl. 1, fig. 5, and the remaining examples are about to begin migrating to the ganglion. It is also evident from Table 5 that this massive proliferation forms a significant fraction of the total number of placodal cells present in any embryo of this stage.

Table 5. *The incidence of large placodal thickenings related to the ophthalmic lobe of the trigeminal ganglion during the final phase of placodal activity. The cell count for each large placodal site is given in brackets.*

Embryo no.	Age (days)	Crown-rump length (mm.)	Total number of placodal cells: Left	Right
C 8	22	8·0	17 (–)	19 (15)
15	—	8·0	86 (25, 25)	86 (16, 25)
4	—	8·0	47 (15)	34 (10)
561	—	8·0	60 (18)	84 (20, 18, 23)
16	—	8·2	54 (–)	41 (20)
17	—	8·2	61 (–)	62 (15)
C 9	—	8·5	25 (10)	5 (–)
18	—	8·5	47 (15)	37 (18)
19	—	8·5	53 (12, 36)	26 (–)
C 13	22	8·7	72 (12, 17)	65 (26, 16)
C 14	22	9·0	60 (20)	75 (19, 20, 16)
C 3	22	9·0	43 (21)	17 (–)
20	—	9.0	69 (22, 25)	31 (–)
25	—	9·0	21 (–)	34 (–)
21	—	9·0	12 (–)	10 (–)
537	—	9·0	—	17 (–)
551	—	9·0	10 (–)	5 (–)
552	—	9·0	22 (–)	—
8	—	9·0	11 (–)	—
C 20	23	9·2	—	5 (–)
C 21	23	9·2	—	—
550	—	9·2	—	—
23	—	9·5	6 (–)	28 (28)
22	—	9·5	—	—

The 9·0 mm. stage marks the end of the phase of active placodal proliferation. Beyond this stage there is a marked decline in the number of placodal thickenings encountered and fewer of these have the form of connecting strands. The epidermis changes to a cubical epithelium which, for a short time, may bear occasional small nodules of rarely more than six cells; these are residual and senile placodal sites which usually disappear without trace by the 9·2 mm. stage. The exception given by the right side of the 9·5 mm. embryo no. 23 is due to the laggard detachment of a large placodal spur related to the ophthalmic lobe of the ganglion; this type of placodal thickening is always the last to disappear.

DISCUSSION

In the sheep the greater part of the Gasserian ganglion originates from the neural crest, but there is convincing evidence that this material is augmented by cells which proliferate from a wide area of the overlying ectoderm. Bartelmez (1924) introduced the term 'area V' to distinguish the same territory in the early human

embryo. Unlike the dorso-lateral and epibranchial placodes, which consist of distinct localized thickenings showing central proliferation, the trigeminal placode has a diffuse form; it appears as isolated and irregular thickenings from which short spurs of cells are proliferated towards the ganglion. During the early phase of placodal activity these thickenings are distributed in an area extending over the whole ganglionic rudiment and also rostral to it, but after the 8 mm. stage they are restricted to the more rostral district beneath which the ophthalmic ramus differentiates.

An examination of these placodal sites in a graded series of embryos reveals three different types of thickening. The first type is a minute cellular nodule of up to twelve cells arranged as a flat cushion, or as a conical papilla, or as a short spur projecting obliquely into the mesenchyme. Such thickenings are produced by local mitotic activity and may be interpreted as sites of impending cellular detachment and migration. In the second type of thickening the epidermal cells traverse the mesenchyme as a continuous strand which fuses intimately with the outer face of the ganglion. These connecting strands represent streams of placodal cells which have been fixed at the moment of their migration and entry into the ganglion. The third type of placodal thickening predominates in the rostral area covering the ophthalmic lobe of the ganglion and is a massive stream of between twenty and thirty-six cells.

Several important conclusions about the pattern of activity of the trigeminal placode may be drawn from the results presented in Table 4. First, there is the marked regularity with which the evidence of epidermal proliferation appears in almost every embryo between the 23-somite and the 9·0 mm. stages. If embryos larger than this may be disregarded, since placodal activity shows a sharp decline after this stage, then it is evident that unmistakable placodal thickenings have been found in eighty-one of eighty-six placodes examined, i.e. in 94 % of the total. It seems reasonable to infer from this high value that the proliferation may be a continuous activity. In this respect the trigeminal placode stands in marked contrast to the epibranchial placodes, certainly of the VII and IX nerves, where the pattern is one of bursts of proliferation alternating with periods of inactivity (Batten, 1954).

Secondly the fact that in every case the number of connecting spurs is fewer than half the total number of placodal sites suggests that each thickening may rest in the epidermis for a short time before it begins to detach cells, and also that once this has started the actual migration is rapidly completed. Additional support for the last idea comes from the fact that only eight examples (9·3 %) were observed of short cellular spurs projecting from the ganglion in such a way as to be accepted as incompletely incorporated placodal contributions. If this interpretation of placodal activity as a process of continuous proliferation followed by intermittent detachment and rapid migration is valid, then one should not expect to find a large number of sites in which a connexion between the ectoderm and the ganglion afforded irrefutable evidence of a placodal contribution. Since, however, one or more connecting spurs are found in fifty-five of the eighty-six placodes examined, representing 63·9 % of the total, the reality of the process can hardly be held in doubt.

The present observations suggest that the peak of activity, as indicated by numerous placodal sites and the high total count of their cells, is reached about the 6–7 mm. stages. During this period the placodal cells emigrate to all parts of the ganglionic rudiment. From the 8 mm. stage onwards the placode is still moderately active, but the placodal sites are now mainly of the massive type which are contributed to the ophthalmic lobe during the phase when the fibrous ophthalmic ramus is developing. The relative importance of these massive contributions to the ophthalmic lobe during the closing phase of placodal activity is expressed in Table 5. Although the trigeminal placode is already active in the 23-somite sheep embryo it is probable that the process may commence at a still earlier stage, as Bartelmez & Evans (1926) and Campenhout (1948) have found in the human embryo.

The discrepancy between the conflicting opinions mentioned in the Introduction involves the evidence for the actual migration of placodal cells into the trigeminal ganglion. Both Holmdahl (1928) and Weigner (1901) have denied the existence of placodal contacts. Adelmann (1925) admits that the trigeminal ganglion in the rat has a broad area of contact with the epidermis, but is firmly convinced of the complete absence of placodal proliferation. In 200 embryos he found only a few cases of epidermal papillae which he explained as fortuitous adhesions of mesenchymal or ganglionic cells. It is perhaps significant that his observations end at the 34-somite stage, which, if the embryos of the rat and sheep may be broadly compared, is only half-way through the period of activity of this placode in the sheep and well before the phase when the ophthalmic lobe receives its major placodal contribution. This fact may explain Adelmann's statement (1925, p. 65) that 'a placodal origin for the ramus ophthalmicus profundus trigemini in the mammal ...is untenable'. While the short spur form of most placodal sites in the sheep may suggest the detachment of single, or at the most a few, cells into the mesenchyme, the only positive evidence of migration is the presence of cellular strands connecting the ectoderm with the ganglion. These connexions are essentially transient features which may not be present in every embryo and, even when present, may easily be overlooked. It must be admitted that the evidence for migration is circumstantial, but nevertheless deserves serious consideration since it occurs repeatedly in a closely graded series of embryos. The most reasonable conclusion is that the short conical thickening of the ectoderm and the long connecting spur represent the extreme stages of a cellular proliferation and migration which is discontinuous both in space and in time.

Except for a few minor points the derivation of the trigeminal placode is fundamentally similar to the descriptions given by Campenhout (1935, 1936, 1937, 1948) for the pig, chick and human, Coërs (1946) for the rabbit and by Halley (1955) for the cat. As in the pig the ventral migration of the placodal field down the surface of the head in the sheep embryo precedes the parallel downgrowth of the neural crest V. Campenhout has attempted to explain this by postulating a chemical induction of placodal sites in the overlying ectoderm by an agent traceable to degenerating nuclear fragments found among the cells of developing ganglion. Similar pycnotic debris has been reported in the cat (Halley, 1955), but despite a careful scrutiny there is no evidence of any cellular degeneration within the trigeminal

ganglion in the sheep during the period of placodal activity. An apparent exception occurs during the 19th day at a time when the ventral border of the neural crest V is becoming clearly delimited from the mesenchyme of the mandibular arch. Similar fleeting evidence of degeneration at this interface has also been recorded by Adelmann (1925) and Davis (1923) in rat and human embryos of the corresponding stage.

The conclusion that both the maxillo-mandibular and the ophthalmic portions of the trigeminal ganglion receive a placodal accession in the sheep is in harmony with the findings of Campenhout (1937, 1948) in the pig and human, and Halley (1955) in the cat. There is, however, no indication of the formation of a separate profundus ganglion of placodal origin as Coërs (1946) found in the rabbit. Yet the several massive placodal sites which contribute to the ophthalmic part of the trigeminal ganglion in the sheep resemble the single site of the rabbit in that they are the last to disappear.

In every vertebrate class the profundus ganglion, and to a lesser extent the Gasserian ganglion, are associated during their development with placodal thickenings, and an excellent review of the comparative development of this nerve is given by Knouff (1927). The peculiar position of the trigeminal placode has misled several writers, notably Campenhout (1936, 1948), Da Costa (1923), and Giglio-Tos (1902), into classifying it as dorso-lateral or even as epibranchial. As early as 1920, however, Landacre pointed out that although the trigeminal nerve contained general somatic sensory fibres it invariably lacked special somatic sensory or lateral line fibres, so that the use of the term 'dorso-lateral placode' was inaccurate. In the same way de Beer (1924) emphasized the incongruity of accepting the profundus placode in the selachian as dorso-lateral when the definitive nerve was known to contain exclusively somatic sensory fibres. Landacre (1920, p. 304) further suggested that the placodal source of the profundus ganglion represented 'the most marked and constant displacement of the neural crest in the head region'. This conforms closely with the opinion of Herrick (1910) that the whole of the lateral surface of the embryonic head is potentially nervous. According to Landacre (1910, 1914, 1920) the general somatic sensory component of the cranial nerves is derived from the neural crest either directly or, in the single exception of the trigeminus, also by later placodal contribution from the overlying ectoderm. Knouff (1927) reached the same conclusion in the frog. Moreover, the experimental studies of Stone (1924, 1928) have clearly demonstrated the functional specificity of the trigeminal placode in *Amblystoma*. And in the chick similar extirpation procedures have yielded evidence of a dual origin of the cells of the trigeminal ganglion (Yntema, 1942).

It is unfortunately not possible to determine the fate of the placodal cells in the mammal after they are incorporated into the trigeminal ganglion owing to the absence of cytological features by which they might be distinguished from crest cells. Since, however, there is in the sheep no evidence of cellular degeneration within the ganglion one may postulate that the placodal cells differentiate either into neuroblasts or into sheath cells. It is significant that the epibranchial placode of the vagus nerve in the sheep provides convincing evidence of the transformation of migrant placodal cells into neuroblasts (Batten, 1956). When the present observations are considered in the light of the embryological and experimental studies

on the Anamniota it seems logical to accept the trigeminal placode of the mammal as a general cutaneous placode, as Ariëns Kappers (1941) and Coërs (1946) have done.

SUMMARY

1. A trigeminal placode is active in sheep embryos from the 23-somite to the 9·2 mm. stages. It is present as an extensive, but ill-defined, ectodermal area overlying and reaching ahead of the developing ganglion.

2. Within this area local proliferation of the epidermis produces small thickenings of up to twelve cells and, more especially in the rostral region, larger thickenings of from twenty to thirty-six cells.

3. The presence of placodal sites in the form of streams of cells connected with the ganglion is accepted as evidence of a placodal contribution to the latter. Placodal activity is visualized as an intermittent process of cellular detachment followed by rapid migration and incorporation into the ganglion.

4. The maxillo-mandibular portion of the trigeminal ganglion arises principally from neural crest V, but receives a limited contribution from placodal thickenings.

5. The ophthalmic lobe of the ganglion similarly has a dual origin, but here the placodal contribution is significantly greater than in the maxillo-mandibular portion.

6. Although there is no certainty as to their exact fate, there is a strong suggestion that the placodal cells received by the trigeminal ganglion differentiate either into sheath cells or into neuroblasts.

This investigation is an extension of part of a thesis submitted for the London Ph.D. degree, under the supervision of Prof. E. C. Amoroso, to whom I wish to express my gratitude for his advice and guidance. I am also grateful to Prof. J. D. Boyd for allowing me access to his valuable collection of sheep embryos and to Dr R. N. Smith for reading and criticising the manuscript.

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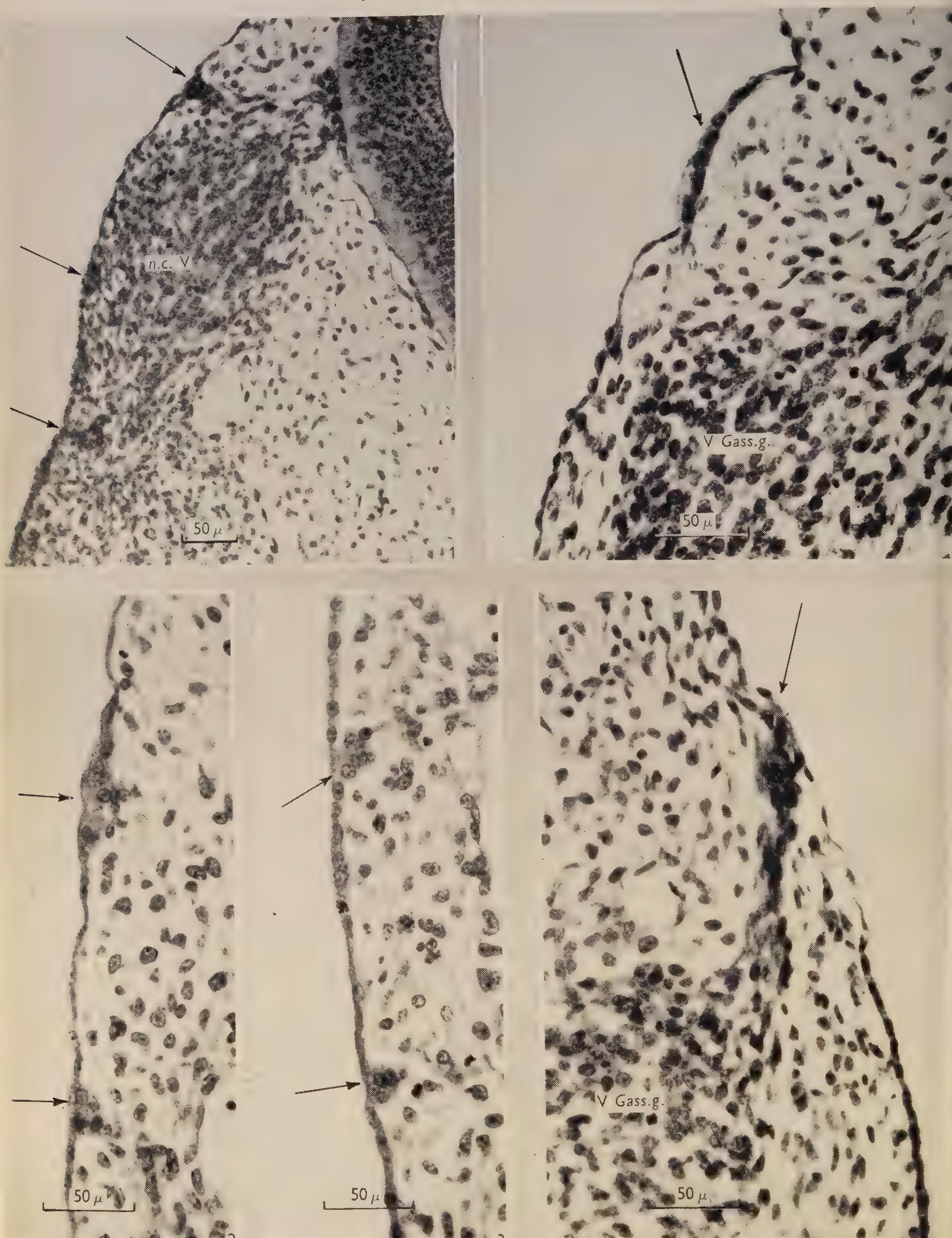

BATTEN—The activity of the trigeminal placode in the sheep embryo

(Facing p. 187)

LIST OF ABBREVIATIONS

A. 1	pharyngeal arch 1	*m.r.*	motor root of mandibular ramus
A. 1 (*max.*)	maxillary bud of arch 1	*n.c.* V	trigeminal neural crest
A. 1 (*mand.*)	mandibular process	*n.c.* VII	facial neural crest
A. 2	pharyngeal arch 2	*ophth.r.*	ophthalmic ramus
cl.pl. 1	closing plate of first pharyngeal pouch	*ophth. lobe*	ophthalmic lobe of Gasserian ganglion
mand.r.	mandibular ramus	*s.r.*	sensory root of trigeminal nerve
max.r.	maxillary ramus	V *Gass.g.*	Gasserian ganglion

EXPLANATION OF PLATE

Plate 1

Fig. 1. Coronal section through the trigeminal ganglion in a 33-somite sheep embryo no. 11 A. The thin epidermis lies close to the ganglion and bears three placodal spurs (arrows).

Fig. 2. Section through the left side of a 32-somite sheep embryo no. 12 showing two placodal sites (arrows) in the ectoderm rostral to the tip of the trigeminal ganglion.

Fig. 3. Section through the right side of the same embryo as fig. 2 showing two similar placodal sites (arrows).

Fig. 4. Section through the trigeminal ganglion in an 8 mm. sheep embryo no. 15. The young neuroblasts are separated from the epidermis by a zone of mesenchyme. A narrow file of eight placodal cells (arrow) is about to detach from the epidermis; this file is site 7 in Text-fig. 3.

Fig. 5. Section through the ophthalmic part of the trigeminal ganglion in the same 8 mm. sheep embryo showing a massive spur of about thirty cells (arrow) streaming into the ganglion. This spur is similar to sites 1 and 2 in Text-fig. 3.

DEVELOPMENT OF THE HUMAN LESSER SAC

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Our knowledge of the normal course of development of the lesser sac is in a confused state and is inadequate to account for some of the congenital anomalies met with in this region. This is perhaps to be attributed to the fact that since the pioneer work of Broman (1904) on the development of the omental bursa in man and in other vertebrates, no serious attempt has been made to reinvestigate the subject.

Müller (1830) considered that the omental bursa was formed as the result of a folding of the dorsal mesogastrium. This interpretation was accepted for the next fifty years till His (1880), Ravn (1889), Mall (1891, 1897, 1910) and Swaen (1896) found that the lesser sac was an independent formation commencing as a small recess on the right side of the foregut tube. Broman (1904), in his extensive monograph on the omental bursa, added details which were lacking in these earlier accounts. Later (1938) he came to the conclusion that the omental bursa of mammals was formed of hepato-enteric and pancreatico-enteric recesses. Vilkari (1950) made a detailed investigation of the infracardiac bursa in man and showed that the bursa persisted in a large proportion of full-term foetuses and might occasionally be found even during adult life.

According to the description in current text-books of embryology, the lesser sac begins its development as a shallow pocket appearing on the right surface of the dorsal mesogastrium. The recess burrows deeper into the thickness of the dorsal mesogastrium and grows in a cranial direction forming the pneumato-enteric recess. The pneumato-enteric recess is then divided by the developing diaphragm into a cranial portion, the infracardiac bursa (Broman, 1904), and a caudal part which becomes the superior recess of the lesser sac. An extension of the pneumato-enteric recess to the left, dorsal to the stomach, results in the formation of the omental bursa proper.

MATERIAL AND METHODS

Twenty-six serially sectioned human embryos ranging from 3 to 99 mm. (C.R.) and one *Hylobates mülleri* foetus 57 mm. (C.R.) were examined during the course of the present investigation. Some additional information was also obtained from the dissection of the lesser sac in a juvenile *H. hoolock*.

For purposes of convenience, the embryos are classified into five age-groups (see Table 1).

Group I: embryos in which the pneumato-enteric recess has not developed (no. examined, 1).

Group II: embryos of 3·8–5 mm. in which the pneumato-enteric recess appears as a longitudinal cleft and does not extend into the dorsal mesogastrium proper (no. examined, 4).

Group III: embryos of 6–12·25 mm. in which the pneumato-enteric recess has invaded the dorsal mesogastrium but the infracardiac bursal portion of the recess remains in continuity with the hepato-enteric part of the pneumato-enteric recess (no. examined, 8).

Group IV: embryos of 13–30 mm. in which the infracardiac bursa has become separate from the hepato-enteric portion of the pneumato-enteric recess. Embryos of this group show marked development of the omental bursa (no. examined, 9).

Group V: embryos of 37–99 mm. in which fusion of the dorsal mesogastrium to the posterior abdominal wall and to the transverse mesocolon is a prominent feature (no. examined, 4).

Table 1. *Embryos examined*

	Serial no.	length (mm.)	Thickness of sections (μ)
Group I	—	3	—
Group II	H. 86	3·8	10
	—	4	—
	—	5	8
	Millard	5	8
Group III	—	6	10
	Barnes	7–8	8
	H. 237	8·5	5
	H. 226 (J.T.W.)	9	10
	H. 116	9–10	10
	Thurkettle	9–10	8
	H. 67 (J. T. W.)	9·5–10	10
	H. 250	12·25	8
Group IV	H. 23	13	10
	H. 24	13·5	10
	H. 241	14	8
	H. 640	17	6
	H. 25	17	10
	H. 242	18	10
	H. 594	22	6
	H. 583	27	6
	H. 180	30	12
Group V	H. 549	37	10
	H. 653	46	7
	H. 183	60	10
	H. 80	99	15

All embryos with a serial number are in the Department of Anatomy, University of Cambridge. The 4, 5 mm. (Millard), 7–8 mm. (Barnes) and 9–10 mm. (Thurkettle) were made available by the Department of Anatomy, Charing Cross Hospital Medical School. Two embryos of 5 and 6 mm. were obtained from the Department of Anatomy and Embryology, University College, London, while the single 3 mm. embryo of the series belongs to the author. All the sections were cut in the transverse plane.

Terminology. In this communication, the terms 'hepato-enteric recess', 'hepatic portion of the pneumato-enteric recess' and 'hepatic portion of the lesser sac' are used synonymously to denote that part of the cavity of the lesser sac which is related to the caudate lobe of the liver. This cavity, in later development, forms the superior recess of the lesser sac. The term 'omental bursa proper' or 'omental portion of the lesser sac' refers to that part of the lesser sac which lies dorsal to the stomach and

is continued into the free caudal portion of the dorsal mesogastrium. The term 'pancreatico-enteric recess' is applied to a small portion of the lesser sac between the caudal part of the hepato-enteric recess and the cranial portion of the omental bursa proper. At times this juctional region is indistinct and in these cases the pancreatico-enteric recess appears as a part of the omental bursa proper. The 'pneumato-enteric recess' refers to the cavity of the lesser sac during its early development. It consists of a cranial infra-cardiac bursa and a caudal hepato-enteric recess, both of which remain in direct continuity until their separation occurs about the 13 mm. stage.

OBSERVATIONS

A 'type specimen' belonging to each of the groups II, III and IV is described in some detail with brief notes on the individual embryos of groups II and III. A general summary of the embryos of group IV and a few relevant facts with regard to embryos of group V are also included in the present observations.

Group I. 3 mm. embryo

The single embryo belonging to this group represents such an early stage of development that no anlage of the right pneumato-enteric recess is noticeable.

Group II. 3·8–5 mm. embryos (type specimen, 3·8 mm.)

The cranial end of the right pneumato-enteric recess lies between the ventral part of the oesophagus and the right lung bud. The ventral portion of the recess is very narrow and is lined by a single layer of epithelium. When traced in the caudal direction, the recess gradually extends dorsally and comes to lie lateral to the oesophagus. Between 20 and 100μ from the cranial end, the recess is dilated in its dorsal half while the ventral half remains narrow as at the commencement (Pl. 1, fig. 1). Between 100 and 120μ from the cranial end, the recess is dilated in its central portion while the dorsal and ventral extremities remain narrow. Further caudally, the dorsal part of the recess becomes dilated once again, and finally this dilated dorsal segment opens into the peritoneal cavity (Pl. 1, fig. 2). The total length of the right pneumato-enteric recess is 170μ. The left pneumato-enteric recess is not present in this embryo.

The bar of mesoderm forming the lateral wall of the right pneumato-enteric recess blends cranially and ventrally with the mesodermal anlage of the right lung bud, while caudally it is split into dorsal and ventral lips by the opening of the recess into the general peritoneal cavity (Pl. 1, fig. 2). The ventral lip of mesoderm guarding the foramen blends caudally with the septum transversum while the dorsal lip merges with the dorsal mesogastrium.

The entodermal anlage of the stomach forms the dilated segment of the foregut. The right surface of the stomach is flattened and is directed slightly dorsally while its left surface is convex and faces ventrally. The dorsal mesogastrium forms a thick mid-line mesentery at this stage.

The ventral mesogastrium is very short and sagittally placed (Pl. 1, fig. 2). In the region of the duodenum, the mesoderm of the septum transversum containing the

liver is broadly in apposition with the lateral walls of the duodenal tube so that there is no ventral mesentery at this place (Pl. 1, fig. 3).

The pneumato-enteric recess is lined by a single layer of epithelium in its narrow cranial portion, while the lining becomes several layers deep in its caudal half. This increase in the layers of cells is particularly noticeable opposite the dilated segments of the recess.

4 mm. embryo. The cranial extremity of the right pneumato-enteric recess lies ventrolateral to the oesophagus and caudal to the entodermal anlage of the right lung bud. The recess soon passes dorsally to lie directly lateral to the oesophagus. Further caudally, the recess communicates with the general peritoneal cavity. The ventral lip of mesoderm guarding the foramen is thin and short, while the dorsal lip is thick and long. The cavity of the pneumato-enteric recess is of uniform width except caudally where the ventral part of the recess is dilated. The entodermal anlage of the stomach tube is flattened from side to side without any definite bulging of its left surface.

5 mm. embryo. The right pneumato-enteric recess is only 56μ in length and is placed between the entodermal anlage of the right lung bud and the oesophagus. The opening of the recess into the pleuroperitoneal coelom is guarded by a large ventral and a rudimentary dorsal lip of mesoderm. The entodermal anlage of the stomach in transverse section resembles that of the 4 mm. embryo.

5 mm. (Millard) embryo. There is a constriction of the central portion of the right pneumato-entéric recess in its cranial part so that sections passing through this region show a small dorsal and a larger ventral cavity (Pl. 1, fig. 4). Further caudally, the cavity of the recess extends dorsal to the stomach which in this region shows a flattened dorsal surface directed partly towards the right while its ventral surface is convex and faces slightly to the left.

The length of the recess is 304μ and the opening of the recess into the general peritoneal cavity is indefinite since the ventral lip guarding the orifice is now invaded by liver tissue and is in contact with the dorsal lip (Pl. 2, fig. 5).

The left pneumato-enteric recess is not present in any embryo of this group.

Group III. 6–12·25 *mm. embryos* (*type-specimen*, 8·5 *mm.*)

The right pneumato-enteric recess lies ventrolateral to the oesophagus and is separated from the anlage of the right lung bud by the cranial portion of the caval fold, the plica mediastino-pulmonalis. The infracardiac bursa which forms the cranial part of the pneumato-enteric recess is 270μ long. The caudal limit of the infracardiac bursa is arbitrarily chosen to correspond with the caudal end of the entodermal lung bud.

The infracardiac bursa passes without interruption into the hepato-enteric recess. The ventral part of this recess, which lies adjacent to the caudate lobe of the liver, is constricted while the dorsal portion of the hepato-enteric recess adjacent to the caval fold is dilated. The caval fold to a large degree remains free of liver tissue, although the caudate lobe of the liver is clearly recognizable. The inferior vena cava has not yet differentiated inside the caval fold.

The omental portion of the lesser sac begins about 670μ from the cranial extremity of the pneumato-enteric recess and appears as an extension from the dorsal portion

of the hepato-enteric recess. The hepatic and omental portions of the lesser sac are placed almost at right angles to each other and they communicate with the general peritoneal cavity by a common orifice, the foramen of Winslow. This opening is 22 μ long in this embryo, and is directed towards the right mesonephros. The foramen is bounded ventrally by the liver, dorsally by the dorsal lip of the caval fold, cranially by the caudate lobe and caudally by a mesodermal fold extending from the proximal part of the duodenum to the dorsal body wall. This fold contains the hepatic artery and is therefore known as the plica arteriae hepaticae (Broman, 1904).

In transverse section, the anlage of the stomach appears as a flattened tube with dorsal and ventral surfaces. The dorsal mesogastrium is extremely thick and is attached along the mid-line of the dorsal abdominal wall. The most dilated portion of the cavity of the lesser sac is contained within the part of the dorsal mesogastrium lying caudal to the stomach.

The ventral mesogastrium is short and sagittally placed in its cranial part. It gradually becomes oblique and finally transverse when traced in a caudal direction. In the oblique and transverse portion, the ventral mesogastrium is extremely thick. The thick ventral mesentery of the proximal part of the duodenum and the corresponding part of the dorsal mesoduodenum are fused together and are connected with the dorsal surface of the liver.

6 *mm. embryo.* The cranial end of the right pneumato-enteric recess consists of a dilated ventral and a narrow dorsal portion (Pl. 2, fig.6). The caval fold which forms the lateral wall of the recess is partly invaded by liver tissue so that the caudal part of the recess lies adjacent to the liver. The anlage of the omental bursa proper is beginning to differentiate inside the dorsal mesogastrium and is in direct communication with the hepatic portion of the pneumato-enteric recess. The hepatic and the omental portions of the lesser sac communicate with the general peritoneal cavity through a common opening, the foramen of Winslow which is 150 μ long. The length of the infracardiac bursa is 72 μ.

7–8 *mm.* (*Barnes*) *embryo.* This embryo closely resembles the type-specimen of this group. The infracardiac bursa measures 260 μ in its cranio-caudal extent. The entodermal lining of the cranial part of the stomach is rectangular in section with dorsal and ventral mesogastria attached to the dorsal and ventral ends of the right surface of the stomach.

9 *mm. embryo.* The central portion of the hepato-enteric recess is completely occluded leaving a small ventral and a larger dorsal cavity (Pl. 2, fig. 7). The length of the infracardiac bursa is 200 μ.

9–10 *mm. embryo.* The infracardiac bursa which is 360 μ long continues caudally into the hepato-enteric recess without interruption. The caudal part of the hepato-enteric recess becomes subdivided by the plica arteriae hepaticae into a smaller right recess, the cavo-coeliac recess of Broman (1904), and a larger left recess which is prolonged caudally into the omental bursa proper. The cavo-coeliac recess is 80 μ in length and its caudal part opens into the general peritoneal cavity. Consequently, there is no direct communication between the general peritoneal cavity and the lesser sac except through the cavo-coeliac recess. The cavo-coeliac recess has the plica arteriae hepaticae on its left, the caudate lobe on its right, the

ventral mesogastrium ventrally and the inferior vena cava dorsally (Pl. 4, fig. 13).

9–10 *mm.* (*Thurkettle*) *embryo.* In this embryo with situs inversus, both the right and the left pneumato-enteric recesses are present (Pl. 2, fig. 8). The right pneumato-enteric recess is 160μ long and opens caudally into the pleuro-peritoneal coelom.

The left pneumato-enteric recess is larger than the right, and when traced in a caudal direction, the central portion of the recess is occluded while very small cavities persist in the dorsal and ventral parts of the recess. In some sections no trace of the cavity is recognizable even in the dorsal or ventral part. The caudal part of the hepato-enteric recess leads into a poorly developed pancreatico-enteric recess. The omental bursa proper is developed as an independent cavity inside the caudal part of the dorsal mesogastrium (Pl. 3, figs. 10, 11). No portion of the lesser sac has any communication with the general peritoneal cavity. The stomach occupies the median plane and is flattened from side to side (Pl. 3, fig. 9).

12·25 *mm. embryo.* The cavity of the pneumato-enteric recess when traced in a caudal direction shows a narrowing of its lumen before becoming continuous with the cranial end of the hepato-enteric recess. But the infracardiac bursa is not completely separated off from the hepato-enteric recess.

Group IV. 13–30 *mm. embryos* (*type-specimen,* 14 *mm. embryo*)

The infracardiac bursa is not developed in this embryo. The cranial end of the hepato-enteric recess lies between the ventro-lateral aspect of the oesophagus and the caudate lobe of the liver. When traced in a caudal direction, the recess gradually extends dorsally towards the inferior vena cava which is now easily recognizable. Immediately caudal to the caudate lobe, the recess is dilated and further caudally it is subdivided by the plica arterae hepaticae into a right cavo-coeliac recess and left portion which continues into the pancreatico-enteric recess. The cavo-coeliac recess, which is 160μ long, opens into the general peritoneal cavity caudal to the free margin of the ventral mesogastrium. The hepato-enteric recess has no direct access into the general peritoneal cavity except through the cavo-coeliac recess.

The junctional region between the hepato-enteric and the pancreatico-enteric recesses forms a narrow neck, caudal to which the pancreatico-enteric recess gradually extends towards the left to become continuous with the omental portion of the lesser sac. The narrow neck leading into the pancreatico-enteric recess is bounded cranially and on the left by the dorsal mesogastrium containing the left gastric artery, while the *plica arteriae hepaticae* forms the caudal and right boundary (Pl. 4, fig. 13).

The cranial portion of the dorsal mesogastrium is short and thick and is attached to the dorsal surface of the stomach close to the future lesser curvature. The attachment continues caudally till the cranial end of the pancreatico-enteric recess, beyond which the mesogastric attachment gradually passes towards the left border of the stomach adjoining the spleen. The attachment of the dorsal mesogastrium to the dorsal wall remains along the mid-line except where the mesogastrium contains the pancreas. At this region, the dorsal mesogastrium appears to arise from the root of the dorsal mesoduodenum.

The ventral mesogastrium is thin and obliquely placed in its cranial portion, while in its caudal parts the ventral mesogastrium assumes a transverse direction. The entodermal anlage of the stomach tube is triangular close to the cardiac orifice, quadrilateral in the cranial portion of the body, and cylindrical in the caudal part of the body of the stomach (Text-fig. 1).

Summary of the findings in other embryos of group IV. An infracardiac bursa is present in all embryos with the exception of the 22 and 30 mm. embryos in which a bursa is completely lacking. The length of the infracardiac bursa ranges from 696μ in the 27 mm. embryo to 150μ in the smallest (13 mm.) embryo of this group. However, there is no strict correlation between the length of the embryo and the length of the infracardiac bursa; for instance, the bursa is 210μ long in the 17 mm. embryo (H. 25), while its length is 250μ in the 13·5 mm. embryo.

The *plica mediastino-pulmonalis* extends between the anlage of the diaphragm ventrally and the mediastinum dorsally (Pl. 3, fig. 12). In the different embryos of this group, this plica shows considerable variation in thickness, in dorso-ventral length and in its relationship to the root of the right lung.

In the 13·5 mm. embryo, the dorsal two-thirds of the hepato-enteric recess is obliterated over a considerable distance with the cavity persisting only in the ventral part of the recess. In the 30 mm. embryo, the entire cranial portion of the hepato-enteric recess is undeveloped and there is mesodermal tissue connecting the caudate lobe not only with the stomach but also with the dorsal mesogastrium. The hepato-enteric recess in most embryos of this group communicates with the general peritoneal cavity by means of a cavo-coeliac recess; but, in the 13, 22 and 27 mm. embryos, there is a direct communication between the hepato-enteric recess and the general peritoneal cavity.

The junctional region between the hepato-enteric and the pancreatico-enteric recesses forms a narrow neck which usually lies between the left gastric and hepatic arteries. The pancreatico-enteric recess leads caudally into the omental bursa proper which in a majority of embryos of this group shows considerable increase in size by extension of the bursal cavity in a left and caudal direction. But in the 13·5 mm. embryo, and more particularly in the 30 mm. embryo, the cavity of the omental bursa is very narrow and in places completely occluded, with the result that considerable mesodermal connexions exist between the dorsal mesogastrium and the dorsal surface of the stomach (Pl. 4, fig. 16).

The cranial part of the dorsal mesogastrium is attached to the dorsal surface of the stomach and lies close to the ventral mesogastric attachment to the stomach, i.e. close to the future lesser curvature. When traced in a caudal direction, the dorsal mesogastric attachment is gradually shifted towards the left border of the stomach adjoining the spleen. But in the 18 mm. embryo, this shift of the mesogastric attachment from the dorsal surface of the stomach to its extreme left border

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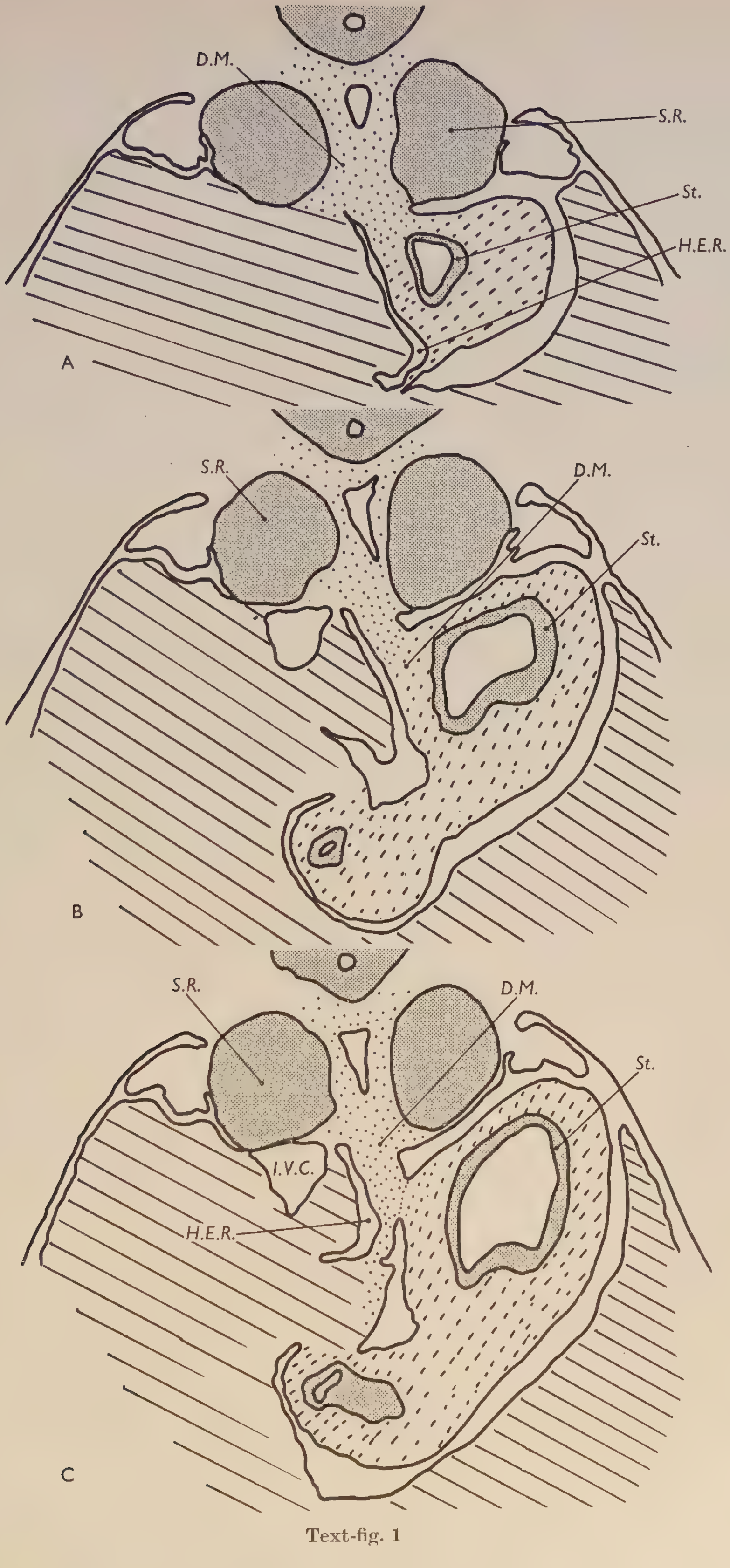
Explanation of Text-fig. 1

Camera lucida drawings ($\times$ 60) of sections through a 14 mm. embryo (H. 241). A, most cranial level; B, intermediate level; C, most caudal level. The drawings indicate that the growth of the endodermal stomach is more marked on its left surface. Note that the dorsal and ventral mesogastria are attached to the dorsal and ventral extremities of the right surface.



Text-fig. 1

is very abrupt and is accomplished within a distance of 40–50μ (Pl. 4, figs. 14, 15).

The root of the dorsal mesogastrium is attached along the median line of the body wall in all embryos of this group. The cranial part of the mesogastrium passing towards the spleen is thin and membranous, while the portion of the mesogastrium between the spleen and the stomach is thick and spongy. In the 17, 27 and 30 mm. embryos the caudal free portion of the mesogastrium is fused with that part of the midgut mesentery which suspends the transverse colon.

The ventral mesogastrium is thinner and longer than in embryos of group III, and the caudal part of the ventral mesogastrium forms the ventral boundary of the cavo-coeliac recess. The free margin of the ventral mesogastrium is directed caudally in all embryos of this group except the 13, 22 and 27 mm. embryos in which the free margin runs caudally and dorsally from the liver towards the proximal part of the duodenum.

Group V. 37–99 *mm. embryos*

The cavity of the infracardiac bursa is poorly developed in the 37 mm. embryo, although the bursa has a cranio-caudal length of 550μ. In the 46 mm. embryo, the infracardiac bursa is very much larger than in the preceding embryo and has a total length of 988μ. In both these embryos the infracardiac bursa is interposed between the ventro-lateral part of the oesophagus and the diaphragm and is practically unrelated to the right lung.

The hepato-enteric recess is well developed in all embryos with the exception of the 60 mm. embryo, in which only the dorsal portion of the recess adjacent to the inferior vena cava is present (Pl. 5, fig. 19). In the 37 and 46 mm. embryos the hepatic and omental portions of the lesser sac communicate with the general peritoneal cavity by a common orifice, the foramen of Winslow. In the 60 mm. embryo there is no communication at all between the greater and lesser peritoneal cavities, while in the 99 mm. embryo, the hepato-enteric recess opens into the general peritoneal cavity by means of a cavo-coeliac recess. The free margin of the ventral mesogastrium runs obliquely in a caudal and dorsal direction from the liver towards the proximal part of the duodenum in the 46 mm. and the 99 mm. embryos, but in the 37 mm. embryo the free margin is directed caudally and ventrally towards the duodenum.

The extreme cranial part of the dorsal mesogastrium remains in the median plane in all embryos of this group. The succeeding part of the mesogastrium stretching towards the spleen is partly fused with the dorsal body wall opposite the left suprarenal gland in the 37, 46 (Pl. 5, fig. 17) and 60 mm. embryos. In the 99 mm. embryo the fusion is almost complete except for a small pocket of peritoneal cavity close to the root of the dorsal mesogastrium.

The part of the dorsal mesogastrium containing the pancreas is partly fused with the dorsal abdominal wall in the 60 mm. embryo, and the fusion is complete in the 99 mm. embryo. The mode of fusion in this region is from the head of the pancreas towards its tail end.

The free portion of the dorsal mesogastrium forming the left wall of the omental bursa is thick and spongy, while the remaining portion of the mesogastrium is thin

and membranous. The transverse mesocolon, which is a part of the midgut mesentery, shows further progress in its fusion with the dorsal mesogastrium than what has been observed in embryos of group IV.

DISCUSSION

Mode of formation of the cavity of the lesser sac

Ravn (1889) believed that the pneumato-enteric recess (*recessus superior sacci omenti*) was formed from the medial portion of the pleuro-peritoneal coelom (*saccus parietalis dorsalis*) which was cut off from the remaining portion of the coelom by a mesodermal fold. This fold, according to Ravn's description and diagrams, appears to take origin from the septum transversum and pass dorsally towards the dorsal mesentery of the foregut. Ravn was also of the opinion that the fusion of the fold to the dorsal mesentery progressed in a cranial direction. This view of a progressive fusion of the fold in a cranial direction cannot be accepted since it would entail a communication between the cranial portion of the pneumato-enteric recess and the pleuro-peritoneal coelom at some stage of development. Such a communication never exists and, on the contrary, a communication is usually present between the caudal portion of the pneumato-enteric recess and the pleuro-peritoneal coelom.

According to the descriptions available in current text-books of embryology (Frazer, 1947; Hamilton, Boyd & Mossman, 1952; Arey, 1954) the pneumato-enteric recess starts as a small pocket on the right surface of the dorsal mesogastrium. This pocket proceeds to burrow deeper into the mesogastrium, and subsequently it grows in a cranial direction towards the root of the right lung bud, forming the pneumato-enteric recess. Growth of this recess towards the left and dorsal to the stomach results in the formation of the omental bursa proper. It is, however, difficult to understand why the lesser sac, which is a part of the general peritoneal cavity, should adopt this different and unique mode of development, while the remaining portion of the peritoneal coelom is formed by the fusion of small clefts appearing in the lateral sheet mesoderm. Further, the current text-book description does not explain how portions of the pneumato-enteric recess can be almost completely obliterated, while other portions of the recess remain dilated as in the 5 mm. (Millard) and 9 mm. embryos (Pl. 2, fig. 7). In the 30 and 60 mm. embryos a communication between the cavity of the lesser sac and the general peritoneal cavity is totally lacking. This finding provides further evidence against the current view, unless one postulates that the initial communication between the pneumato-enteric recess and the general coelom has subsequently become closed in the 30 and 60 mm. embryos. The text-book accounts are also inadequate for the explanation of some of the anomalies of the lesser sac.

The view suggested here is that the pneumato-enteric recess develops in a manner similar to the rest of the coelom by the coalescence of separate clefts appearing in the mesoderm lying lateral to the entodermal anlage of the foregut tube. Usually the formation and fusion of the clefts must be so rapid that a continuous cavity results, but sometimes there is an arrest of development as in the 9 mm. embryo of this series. The tendency of the pneumato-enteric recess to communicate with the general pleuro-peritoneal coelom is understandable since the coelomic clefts them-

selves exhibit a similar tendency to coalesce during their formation. The omental portion of the lesser sac develops independently in the substance of the dorsal mesogastrium proper, but normally it soon establishes continuity with the pneumato-enteric recess. The impression is thus given that the omental portion of the lesser sac develops as an extension from the pneumato-enteric recess. Evidence for the independent origin of the omental portion of the lesser sac is furnished by the 9–10 mm. (Thurkettle) and the 30 mm. embryos, which obviously exhibit an arrest in the fusion process, and in which the principal subdivisions of the lesser sac remain separate. In the embryos examined, the portion of the hepato-enteric recess lodging the caudate process of the liver and also the left and caudal portions of the omental bursa proper form large cavities, but in the junctional regions between the subdivisions of the lesser sac the cavity is poorly developed or even absent. The failure of cavity formation results in the so-called 'adhesions' between organs normally separated by the cavity of the lesser sac.

Caval fold. In the 3·8, 4 and 5 mm. embryos, the pneumato-enteric recess is a semilunar cleft running longitudinally in relation to the ventro-lateral and lateral surfaces of the foregut tube. No part of the recess extends farther dorsally than the dorsal limit of the entodermal gut tube, that is, there is no extension into the dorsal mesogastrium proper. The pneumato-enteric recess at this stage is bounded laterally by the caval fold. The caval fold blends cranially and ventrally with the mesodermal anlage of the right lung bud, while dorsally the fold joins the dorsal mesentery of the foregut. In the ventral and caudal direction the fold is connected with the mesoderm of the septum transversum. At the level of the foramen of Winslow the fold is split into dorsal and ventral lips, the ventral lip being very much the larger of the two. The foramen of Winslow may sometimes be in a more dorsal position than normal, and the dorsal lip is then very small or even absent as in one of the 5 mm. embryos. The mesoderm of the caval fold is of doubtful origin. Descriptively it would be the mesoderm forming the right lateral wall of the foregut tube becoming separated from the gut by the formation of the pneumato-enteric recess. But in view of the later potentialities of the caval fold, which forms a portion of the 'septal diaphragm' lateral to the oesophageal hiatus (Wells, 1954), and in view of the fact that the caudate lobe of the liver develops within this fold, the entire caval fold cranial to the foramen of Winslow is best regarded as a derivative of the septal mesoderm. According to this view, the most cranial portion of the caval fold, which forms the mediastino-pulmonary ligament, will also be of septal origin. The migration of mesodermal cells from the septum transversum towards the dorsal mesentery to form the caval fold becomes understandable in view of the anticipated pathway for the inferior vena cava from its subcardinal segment towards the venous end of the heart in the septum transversum. The failure of development of the vena cava on the left probably accounts for the usual failure of a distinct caval fold to develop on that side. The close developmental correlation of the fold and the inferior vena cava is supported by an anomaly recorded by Hochstetter (1888) in a *Salamander maculosa* in which both the vena cava and the right caval fold were absent.

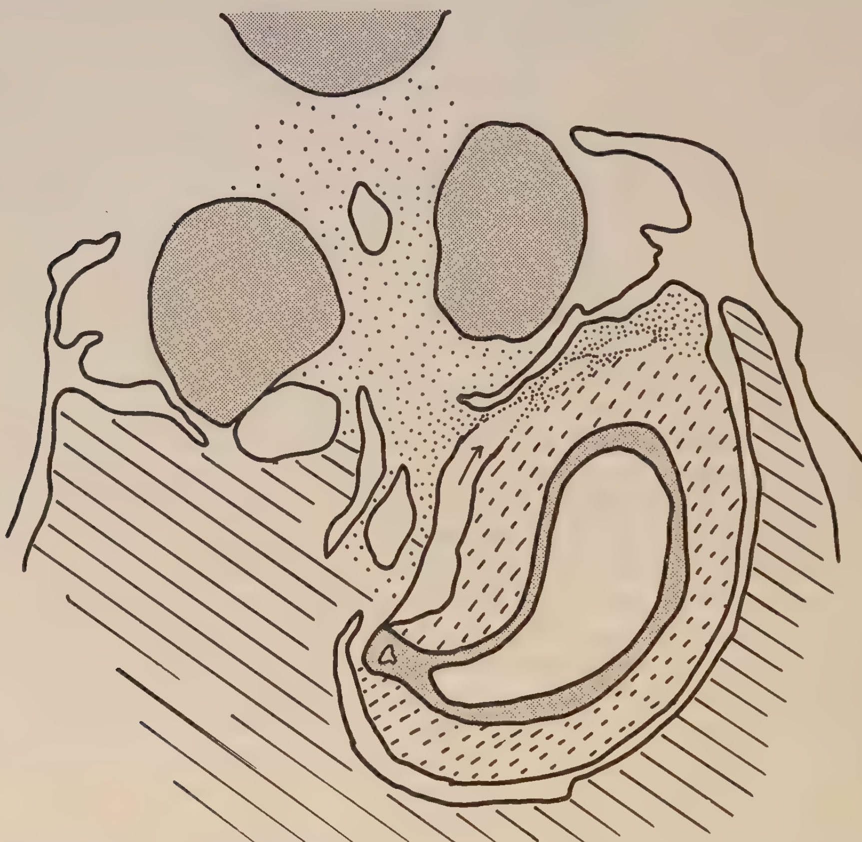
If the view that the caval fold is of septal origin be accepted, then the pneumato-enteric recess is a cavity separating the foregut tube and dorsal mesogastrium from the mesoderm of septal origin in which the whole of the liver, including the caudate

lobe, develops. The cranial portion of the pneumato-enteric recess becomes pinched off by the developing diaphragm at the 13 mm. stage to form the infracardiac bursa. The time of separation of the infracardiac bursa appears to be variable, since Broman (1904) observed that the separation of the bursa was complete in a 11·7 mm. embryo, while Vilkari (1950) found the same in a 12·5 mm. embryo. The 12·25 mm. embryo of the present series shows only a constriction without actual separation of the infracardiac bursa. The portion of the pneumato-enteric recess caudal to the diaphragm remains as the superior recess of the lesser sac. The superior recess has the stomach and dorsal mesogastrium on its left and caudate lobe on its right and ventrally, an arrangement which is retained throughout foetal and post-natal periods.

Rotation of the stomach. It is usually believed that the stomach undergoes a dextro-rotation through an angle of 90° along its longitudinal axis. Enbom (1939) was of opinion that the rotation of the stomach occurred along the dorso-ventral, lateral and sagittal axes; Pernkopf (1922), while denying a mechanical rotation along the longitudinal axis of the stomach, was unable to determine whether the changes in the stomach were due entirely to growth processes or to an actual 'rotation'. The criteria often cited in favour of the theory of rotation are the relative positions of the two gastric nerves, the attachment of the dorsal mesogastrium to the greater curvature of the stomach and the attachment of the ventral mesogastrium to the lesser curvature. If rotation of the stomach is to be judged by the change in position of the gastric nerves, then a similar rotation should also occur in the caudal, intrathoracic portion of the oesophagus, since the vagi have already assumed dorsal and ventral positions in this portion of the gut tube. In embryos of 3·8–22 mm. of this series there is no evidence of rotation of the caudal oesophagus, since this portion of the gut tube shows no change in the shape or orientation of its entodermal lumen which remains compressed from side to side throughout the series. Changes are, however, apparent in the stomach where transverse sections of the entodermal tube of 10–22 mm. embryos reveal an increasing growth and dilatation of the left surface of the stomach, while its right surface remains unaffected (Text-fig. 1A–C). This simple growth process will explain the apparent shift of the ventral mesogastrium towards the right. It may also be added that mere changes in the position of nerves cannot be regarded as proof of rotation.

It is equally erroneous to conclude that the rotation of the stomach occurs because the greater omentum, a derivative of the dorsal mesogastrium, is now attached to the greater curvature (left border) of the stomach. In the 18 mm. embryo the *gastric attachment* of the dorsal mesogastrium, when traced cranio-caudally, changes from the dorsal to the extreme left border of the stomach within a distance of $50\,\mu$ (Pl. 4, figs. 14, 15). If this is interpreted to mean that the dorsal border with its mesogastrium has rotated to become the left border of the stomach within this short distance, the 'rotation' appears to be so drastic that it is doubtful whether it could ever occur in normal development. The developmental changes occurring in the gastric attachment of the dorsal mesogastrium could be effected through an extension of the omental bursa to the left into the compound mesodermal anlage of the stomach wall and mesogastrium where previously no cavity existed (Text-fig. 2). It is perhaps due to these changes in the mesogastric attachment that the right

gastric nerve enters the dorsal surface of the stomach after traversing only the extreme cranial portion of the dorsal mesogastrium; this most cranial part of the mesogastrium is sagittally placed and is hardly affected by the growth of the omental bursa. Cases in which partial or complete septa intervene between the superior and inferior recesses afford further evidence against the dorsal mesogastric attachment as a criterion of rotation. These septa are undoubtedly remnants of the original



Text-fig. 2. Based on a section through a 17 mm. embryo (H. 25). The arrow indicates the direction of extension of the lesser sac into the compound mesoderm of the dorsal mesogastrium and the stomach wall.

dorsal mesogastrium, but they are attached to the dorsal surface of the stomach *close to the lesser curvature*. The mode of formation of the septa will be discussed later.

Boundaries of the foramen of Winslow. In the 3·8 mm. embryo, the foramen of Winslow is bounded by the dorsal and ventral lips of mesoderm which cranially continue into the caval fold (Pl. 1, fig. 2). The foramen has no definite caudal boundary at this stage. The inferior vena cava is later formed inside the dorsal lip while a portion of the right lobe of the liver grows inside the ventral lip. The caval fold is gradually invaded by liver tissue forming the caudate lobe during the 6, 7,

7–8 and 8·5 mm. stages. At the 8·5 mm. stage, the foramen is bounded ventrally by the right lobe of the liver and dorsally by the dorsal lip in which the differentiation of the inferior vena cava has not yet begun. The foramen is bounded cranially by the caval fold containing the caudate lobe of the liver, and caudally by the plica arteriae hepaticae. The foramen at this stage is directed caudally and towards the right so that in transverse sections the cavity of the lesser sac communicates with the general peritoneal cavity, ventral to the right mesonephros.

In the 10–18 mm. embryos there is usually no direct communication between the cavity of the lesser sac and the general peritoneal cavity, the two being connected with each other through a cavo-coeliac recess (Broman, 1904). This recess, which is absent in the 8·5 mm. embryo, is brought into existence by the caudal growth of the caudate lobe of the liver and by the approximation of the right suprarenal gland to the right lobe of the liver. These changes effectively seal off the original communication of the lesser sac with the general peritoneal cavity ventral to the right mesonephros, thereby enclosing a pocket of the peritoneal cavity which is bounded ventrally by the ventral mesogastrium and dorsally by the inferior vena cava. On the left of the cavo-coeliac recess lies the plica arteriae hepaticae, and on its right the caudate lobe (Pl. 4, fig. 13). The cavo-coeliac recess opens into the general peritoneal cavity either ventrally as in the 18 mm. embryo (Pl. 4, fig. 15), or dorsally as in the 17 mm. embryo (H. 640). The mode of communication depends on the extent of the caudal growth of the caudate lobe and on the caudal limit of the ventral mesogastrium.

In the 22 mm. embryo, the hepato-enteric portion of the lesser sac communicates directly with the general peritoneal cavity around an obliquely placed free margin of the ventral mesogastrium, an arrangement which to some extent resembles the adult condition. In this embryo the free margin of the ventral mesogastrium runs obliquely caudally and dorsally from the liver towards the duodenum. The final disposition, whereby the free margin of the ventral mesogastrium faces directly towards the right, is not achieved even in the 99 mm. embryo which in fact shows a cavo-coeliac recess. Although Broman (1904) thought that the cavo-coeliac recess resulted from the formation of the fold of the hepatic artery during the caudal extension of the lesser sac, the recess owes its existence partly at least to the caudal prolongation of the ventral mesogastrium, which characterizes all early embryos.

The growth of the omental bursa and the elongation of the stomach between its relatively fixed cranial and caudal ends produce a flexure of the stomach to the left so that its concave border is now directed towards the right. These changes, along with the rapid growth of the left surface of the stomach, bring about a modification in the disposition of the ventral mesogastrium from the sagittal to the coronal plane. The broad caudal limit of the ventral mesentery, which in the 3·8–6 mm. embryos extends to the region of the umbilicus, is gradually displaced cranially owing to the invasion of the peritoneal cavity ventral to the distal part of the duodenum, where in the immediately previous stages no peritoneal coelom existed. The caudal displacement of the stomach relative to the liver brings about the final change whereby the caudal free margin of the ventral mesogastrium becomes now directed towards the right.

Dorsal mesogastrium and transverse mesocolon. A part of the cranial portion of the dorsal mesogastrium stretching between the spleen and the mid-line has begun to fuse with the dorsal body wall opposite the left suprarenal gland by the 37 mm. stage. The fusion has progressed medially in the 46 and 60 mm. embryos and the fusion becomes almost complete in the 99 mm. embryo, except for a small pocket of peritoneal cavity close to the root of the dorsal mesogastrium. This mode of fusion from lateral to medial is significant, since an arrest of the process may give rise to retro-peritoneal cysts originating from isolated pockets of peritoneal cavity. The portions of the mesogastrium which fuse with the body wall appear to be incorporated with tissues forming the body wall rather than disappear after zygosis. A similar view was expressed by Pernkopf (1922).

The portion of the dorsal mesogastrium containing the pancreas is partly fused with the body wall in the 60 mm. embryo and the fusion is complete in the 99 mm. embryo. The fusion progresses from the medial towards the lateral side, thus contrasting with the cranial, supra-pancreatic portion of the mesogastrium. It is therefore apparent that the only portion of the dorsal mesogastrium to retain its primitive attachment close to the mid-line body wall is the extreme cranial part, which is later converted into the gastrophrenic ligament.

The transverse mesocolon, which is a portion of the midgut mesentery, fuses with the right free margin of the dorsal mesogastrium long before the reduction of the midgut loop from the umbilical coelom. The earliest sign of fusion was observed in the 17 mm. embryo, and the fusion was seen to progress in the 27, 37 (Pl. 5, fig. 18), 46, 60 and 99 mm. embryos. This early fusion perhaps accounts for the fact that in a congenital diaphragmatic hernia through a pleuro-peritoneal opening, herniation of the stomach is found only in those cases in which the tranverse colon herniates into the pleural cavity (Paul & Kanagasuntheram).

Anomalies. According to Weiss (1939) normal and abnormal developments differ neither in principle nor in character. There is always a gradation from the normal to the grossly abnormal. Such gradation indeed exists with regard to the septum which occasionally intervenes between the superior and inferior recesses of the lesser sac. A similar conclusion was reached by Crymble (1913). A complete septum bursarum is unrecorded for any mammal except the gibbon (Kanagasuntheram, 1954). Even in the gibbon a complete septum appears to be inconstant, for recent examination of a 57 mm. *H. mulleri* foetus and of a juvenile *H. hoolock* revealed in both a narrow slit-like communication between the hepato-enteric and the pancreatico-enteric portions of the lesser sac. In the gibbon foetus, the dorsal mesogastrium continued to be attached close to the lesser curvature of the stomach up to the junction of the body and pyloric regions as in the juvenile hoolock gibbon. A similar mode of attachment of the dorsal mesogastrium was observed in the 18 mm. human embryo, and it is clear that if this condition were to persist it would give rise to a partial septum with a narrow orifice of communication between the hepato-enteric and the pancreatico-enteric portions of the lesser sac. A complete septum owes its origin to the fact that the omental bursa proper could be formed independently within the mesoderm of the dorsal mesogastrium as in the 9–10 mm. (Thurkettle) and 30 mm. embryos Various gastric adhesions described by Reid (1913) are undoubtedly remnants of the mesogastrium.

If an independently formed pneumato-enteric recess fails to establish communication with the general peritoneal coelom, the foramen of Winslow will be lacking as in the 9–10, 30 and 60 mm. embryos described in this communication. A caudally directed foramen of Winslow, with a cavo-coeliac recess, is due to the persistence of the embryonic ventral mesentery beyond the proximal part of the duodenum. Fusion of the greater and lesser omenta to the liver and gall bladder represents an arrested phase of development and resembles the condition seen in the 17 mm. embryo. Various other minor adhesions, such as those between the caudate lobe and the stomach, between the caudate process and pancreas, or between the liver and the ventral surface of the stomach, are present in some of the embryos examined. These probably owe their origin to irregularities in the formation of the greater and lesser peritoneal cavities. Absence of fusion between the greater omentum and the transverse mesocolon which is normal for lower mammals is an arrest of development in man.

The fusion of the descending mesocolon to the posterior abdominal wall follows the same pattern as that of the supra-pancreatic portion of the dorsal mesogastrium, the fusion progressing from lateral to medial (Pl. 5, fig. 20). These findings contradict the usual belief that the fusion of the mesocolon progresses from the medial to the lateral side (Arey, 1954).

Mesodermal connections were observed between the lungs and the thoracic wall in a number of embryos, and it is therefore probable that at least some of the pleural adhesions met with in the post-mortem room are of congenital origin.

SUMMARY

The cavity of the lesser sac develops in a manner similar to the rest of the coelom by the coalescence of clefts which appear in the mesoderm lying lateral and dorsal to the caudal part of the foregut tube. The formation and fusion of these clefts are so rapid that a continuous cavity usually results.

Reasons have been adduced to show that the mesoderm of the caval fold may be of septal origin.

The position of the gastric nerves and the attachment of the dorsal mesogastrium to the greater curvature of the stomach cannot be regarded as reliable criteria in favour of a rotation of the stomach. Most changes in the stomach are explicable on simple growth processes assisted by enlargement of the cavity of the lesser sac.

Fusion of the dorsal mesogastrium to the transverse mesocolon begins long before the reduction of the midgut loop from the umbilical coelom.

Fusion of the cranial part of the dorsal mesogastrium to the posterior abdominal wall proceeds from the splenic end towards the root of the dorsal mesogastrium.

Most anomalies, including the formation of partial and complete septa in the lesser sac, are due to an arrest of fusion of the coelomic clefts which normally coalesce to form the cavity of the lesser sac.

I am much indebted to Prof. J. D. Boyd for granting me the facilities to work in his laboratory and for his continued interest and assistance throughout this work. I wish to thank Prof. W. J. Hamilton and Dr M. Abercrombie for the loan of a part

of the material used in the present investigation. My thanks are also due to my colleague, Dr M. Amerasinghe, who was kind enough to show me his dissection of the lesser sac in a full-term human foetus with a complete septum bursarum. I am grateful to Mr J. F. Crane and Mr K. W. Iles for their technical assistance.

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KEY TO LETTERING

B.D.	Bile duct
C.C.R.	Cavo-coeliac recess
C.L.	Caudate lobe of liver
D.A.	Dorsal aorta
D.C.	Descending colon
D.M.	Dorsal mesogastrium
D.M.C.	Descending mesocolon
D.P.	Dorsal pancreatic bud
F.G.	Foregut tube
H.E.R.	Hepato-enteric recess
I.C.B.	Infra-cardiac bursa
I.V.C.	Inferior vena cava
K.	Kidney
L.	Liver
L.S.	Cavity of lesser sac
Lt.L.B.	Left lung bud
Lt.Pn.R.	Left pneumato-enteric recess
Lt.V.	Left vagus nerve
M.G.M.	Midgut mesentery
M.L.	Mesodermal lips
Oe.	Oesophagus
P.	Pancreas
Pl.A.H.	Plica arteriae hepaticae
P.P.C.	Pocket of peritoneal cavity
P.V.	Portal vein
Rt.L.B.	Right lung bud
Rt.Pn.R.	Right pneumato-enteric recess
S.	Spleen
S.F.	Site of fusion of *D.M.* and *M.G.M.*
S.R.	Supra-renal gland
St.	Stomach

EXPLANATION OF PLATES

Plate 1

Fig. 1. Transverse section through the cranial part of the right pneumato-enteric recess of the 3·8 mm. embryo. ×84.

Fig. 2. Transverse section at the level of the opening of the right pneumato-enteric recess into the general peritoneal coelom in the 3·8 mm. embryo. ×84.

Fig. 3. Transverse section through the dorsal pancreatic bud of the 3·8 mm. embryo. Note wide apposition of the liver and septal mesoderm with the ventral and lateral walls of the duodenal tube. ×84.

Fig. 4. Transverse section through the cranial part of the right pneumato-enteric recess of the 5 mm. (Millard) embryo. ×245.

Plate 2

Fig. 5. Transverse section through the caudal part of the right pneumato-enteric recess of the 5 mm. (Millard) embryo. Note apposition of the liver to the dorsal mesogastrium closing the foramen of Winslow. ×180.

Fig. 6. Transverse section through the cranial end of the right pneumato-enteric recess of the 6 mm. embryo. ×180.

Fig. 7. Transverse section from the 9 mm. embryo showing obliteration of the central portion of the hepato-enteric recess. ×196.

Fig. 8. Transverse section from the 9–10 mm. (Thurkettle) embryo showing double pneumato-enteric recesses. ×130.

Plate 3

Fig. 9. Transverse section through the cavity of the lesser sac of the 9–10 mm. (Thurkettle) embryo. Note that the lesser sac lies on the left side owing to a situs inversus. ×150

Fig. 10. Transeverse section through the caudal part of the lesser sac of the 9–10 mm. (Thurkettle) embryo. Note an almost complete absence of the cavity of the lesser sac. ×150

Fig. 11. Transverse section through the free portion of the dorsal mesogastrium of the 9–10 mm. (Thurkettle) embryo. Note that the cavity of the lesser sac is larger than in the preceding section. ×150.

Fig. 12. Transverse section through the infra-cardiac bursa of the 18 mm. embryo. ×196.

PLATE 4

Fig. 13. Transverse section through the junctional region between the hepato-enteric and pancreatico-enteric recesses of the 14 mm. embryo. ×23.

Figs. 14, 15. Transverse sections from the 18 mm. embryo to show the sudden shift of the dorsal mesogastric attachment on the stomach from the dorsal to the extreme left border of the stomach. ×23.

Fig. 16. Transverse section through the region of the lesser sac of the 30 mm. embryo. Note that the cavity of the lesser sac is represented by discrete clefts. ×23.

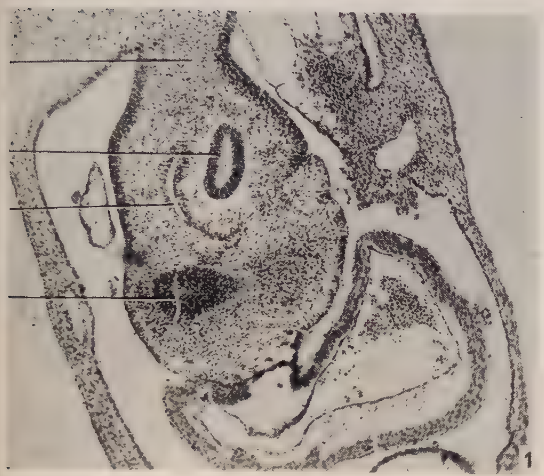
PLATE 5

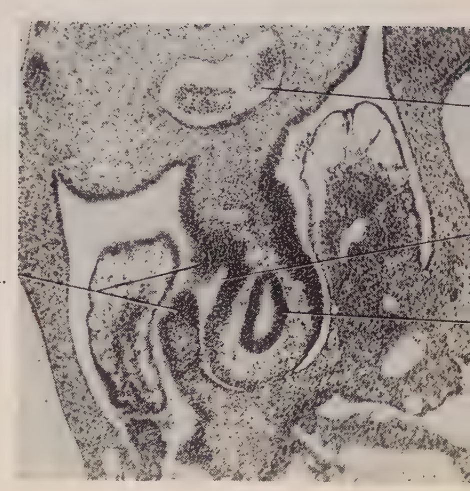
Fig. 17. Transverse section of the 46 mm. embryo showing fusion of the dorsal mesogastrium with the posterior abdominal wall opposite the left suprarenal gland. ×23

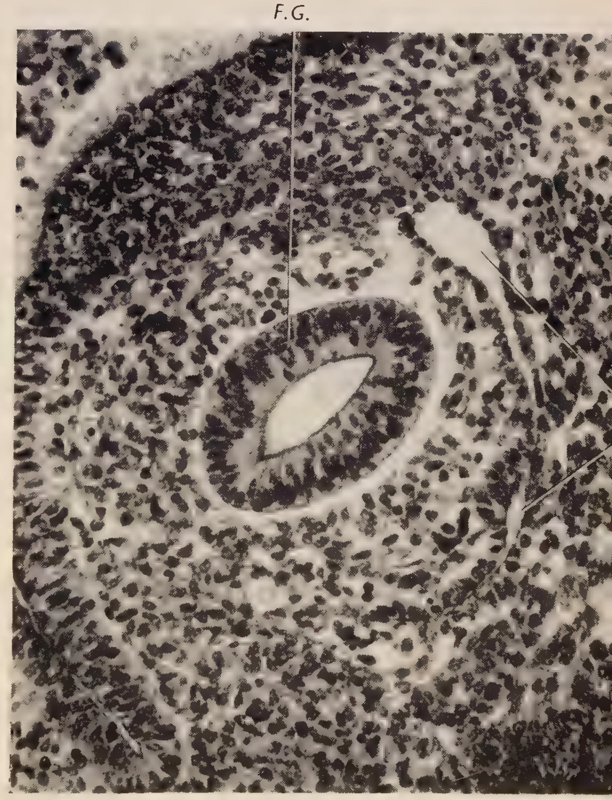
Fig. 18. Transverse section from the 37 mm. embryo showing fusion of the midgut mesentery (transverse mesocolon) with the dorsal mesogastrium. ×23.

Fig. 19. Transverse section through the hepato-enteric recess of the 60 mm. embryo. Note 'adhesions' between caudate lobe of liver and stomach. ×23.

Fig. 20. Transverse section of the 99 mm. embryo showing fusion of the descending mesocolon to the posterior abdominal wall. ×23.







KANAGASUNTHERAM—DEVELOPMENT OF THE HUMAN LESSER SAC

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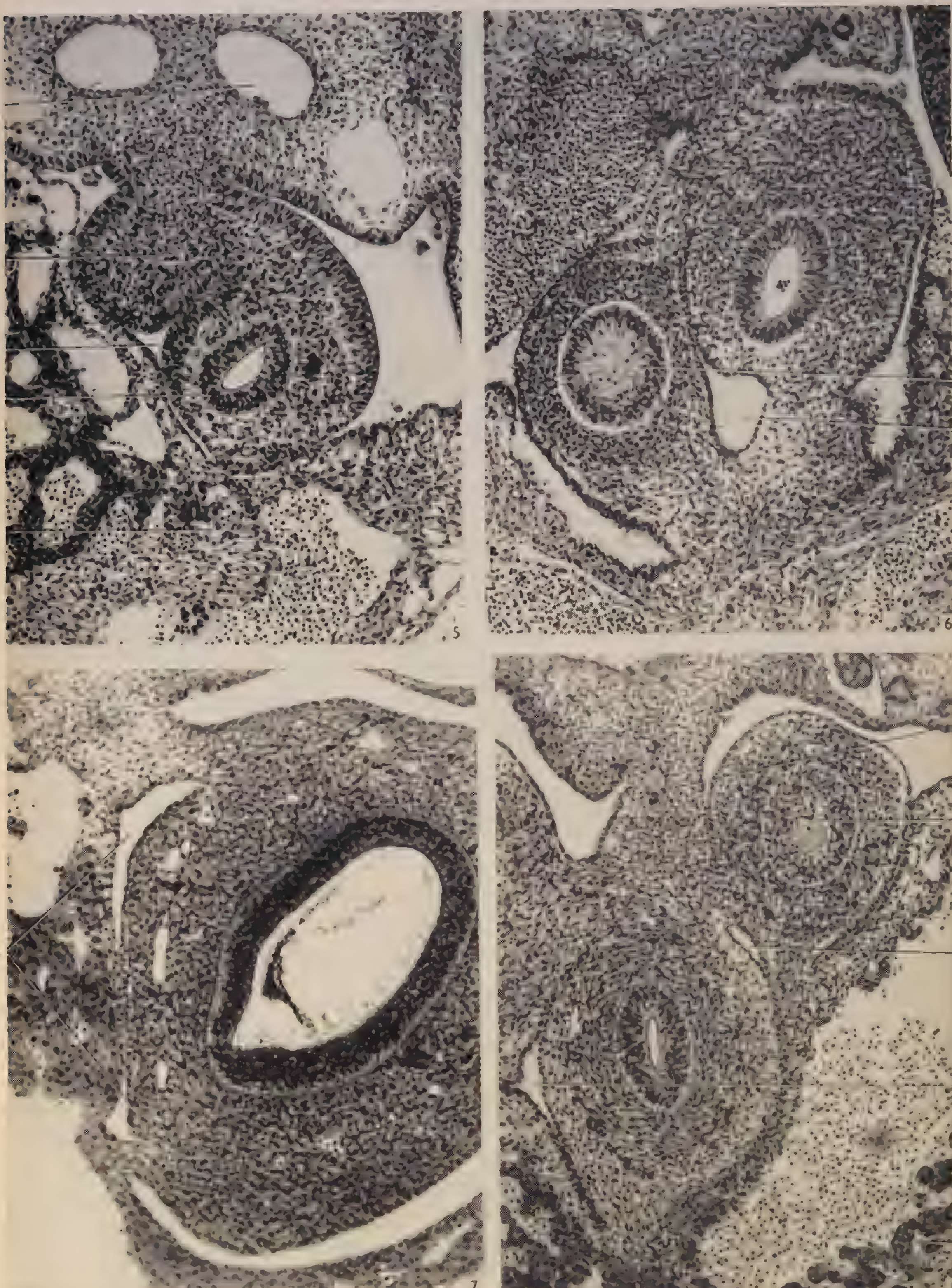

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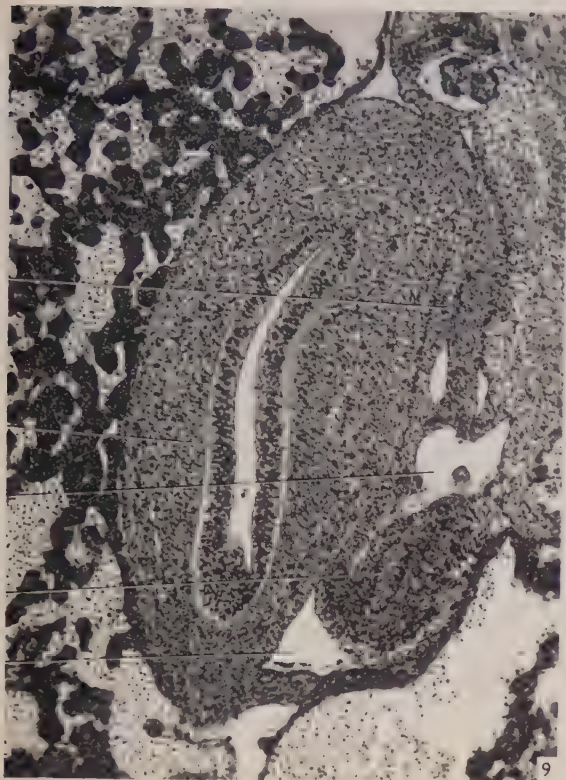

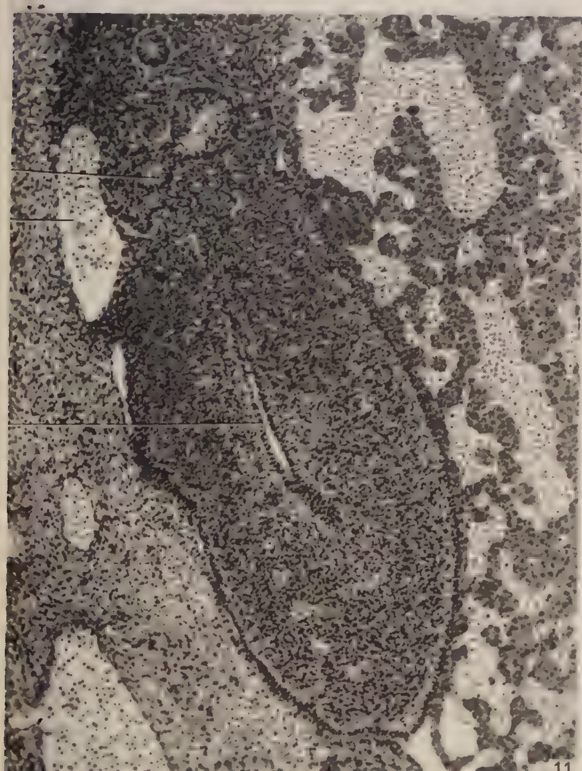

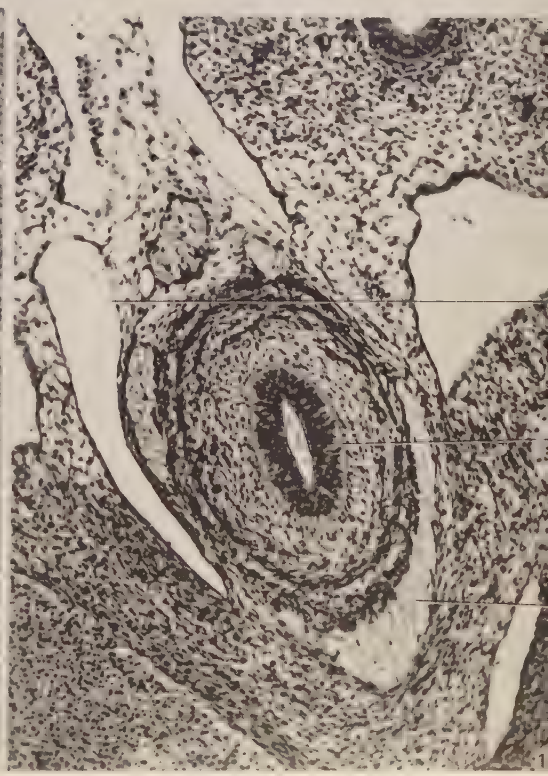

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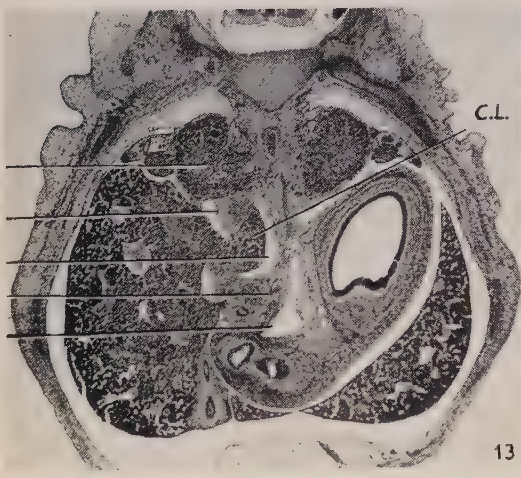

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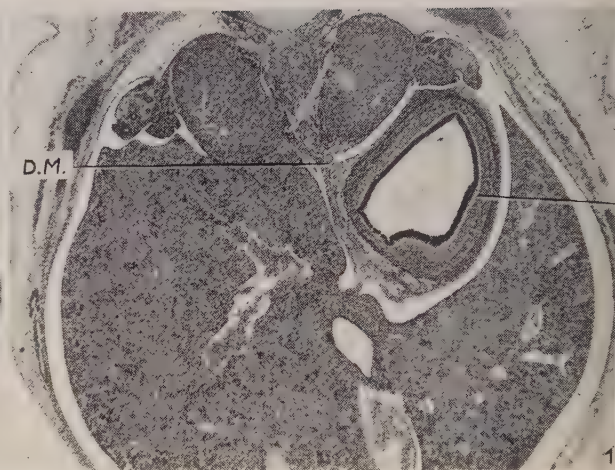

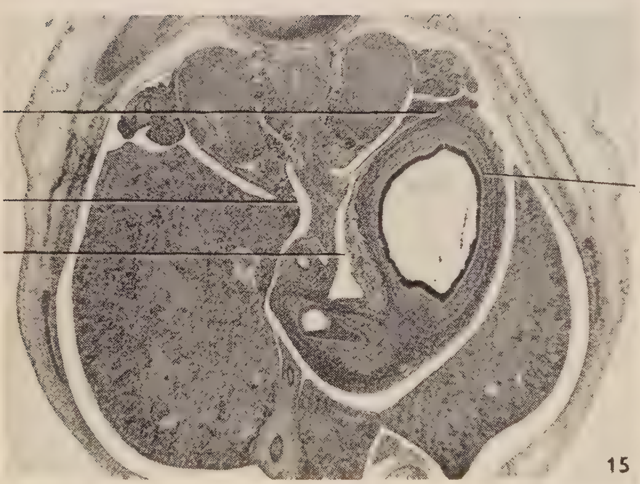

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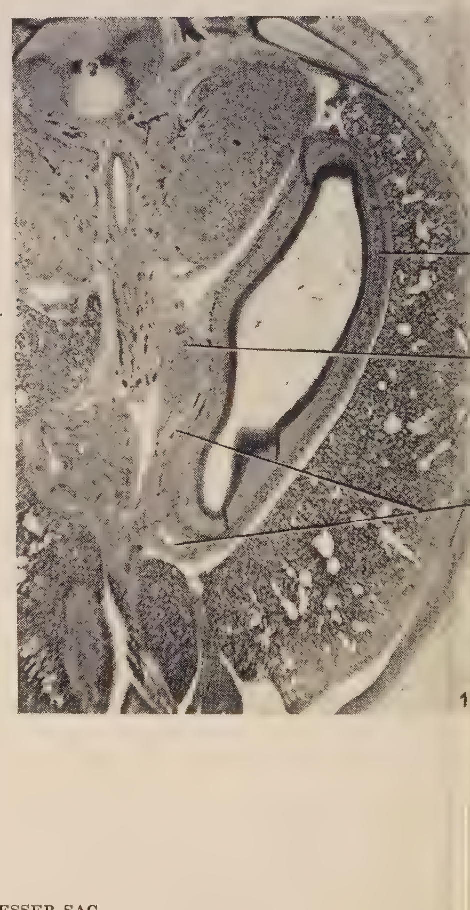
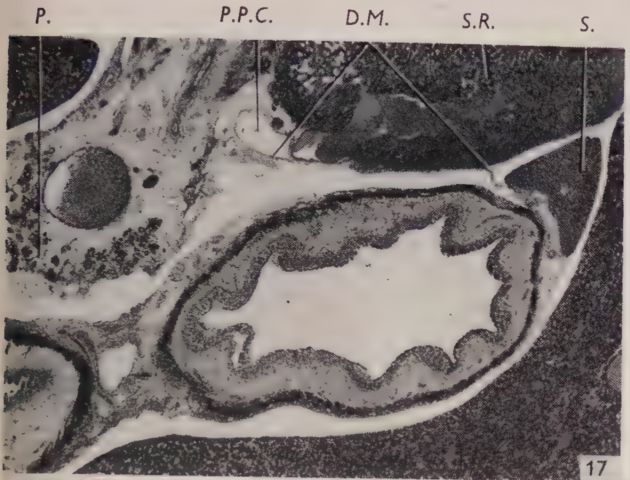


KANAGASUNTHERAM—Development of the human lesser sac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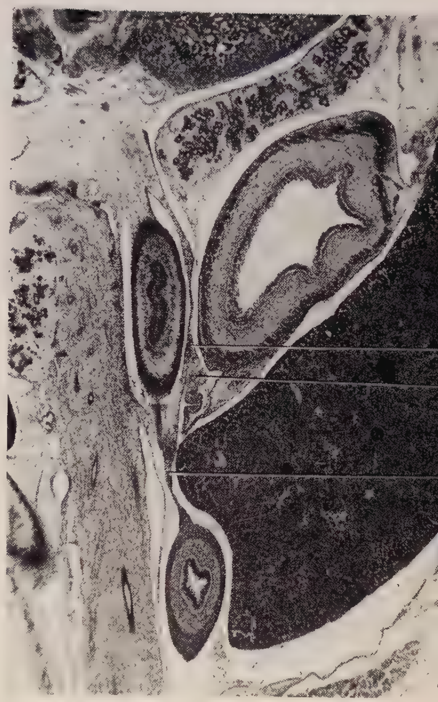

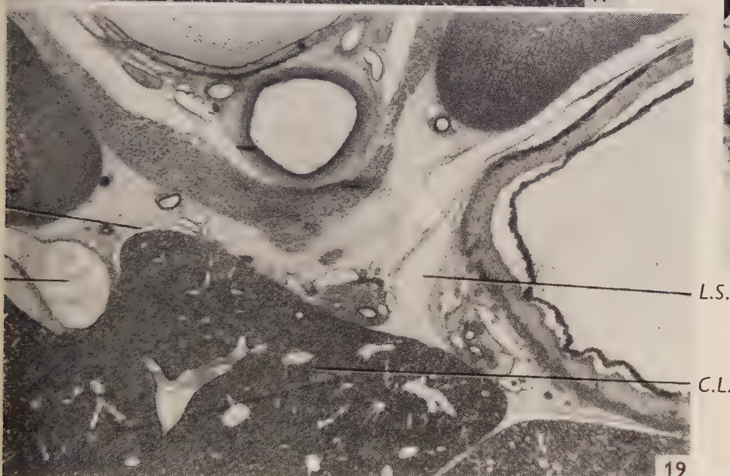
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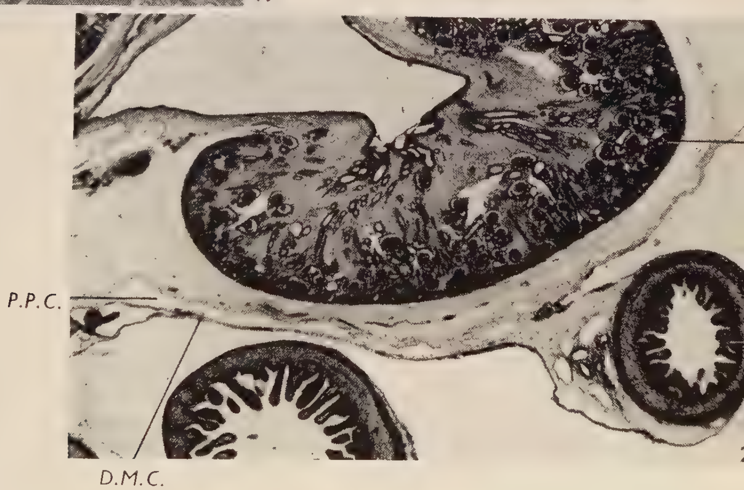
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THE CAROTID SINUS COMPLEX IN THE HEDGEHOG, *ERINACEUS EUROPAEUS*

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In his monograph on the carotid sinus and cerebral circulation Ask-Upmark (1935, p. 58) states that, in *Erinaceus europaeus*, 'the internal (carotid) trunk presents at its origin a most considerable bulbus-like dilatation the caliber of which exceeds that of the common carotid'; and again (p. 59): 'an extremely pronounced dilatation is present at the common origin of the thick internal carotid and the thin occipital artery.'

Brief as it is, this work of Ask-Upmark's is, nevertheless, the first to deal particularly with the bifurcation of *Erinaceus*; it had been mentioned before only in connexion with the general anatomy of the carotids (Otto, 1825; Tandler, 1899; Livini, 1903) although it was, of course, well-known that this is one of the mammals in which the occipital arises from the internal carotid. Besides his observation of the very prominent swelling at the commencement of the internal carotid, Ask-Upmark remarks briefly on the innervation of the region. Thus he says (p. 96): 'this common trunk' (i.e. the internal carotid and occipital) 'was invariably short, dilated, and represented the destination of the arterial sinus nerves, i.e. the sinus.' Of these nerves, the branch from the vagus 'was fairly large-sized' (p. 107) and is 'directed to the bulb below the origin of the occipital artery' (p. 58); there is also a short thick branch from IX to the medial aspect of the bulb, and two short branches from the sympathetic to its posterior surface.

The implication, of course, is that the occipital artery in the hedgehog may enter into the carotid 'reflexogenic zone', which had previously been thought to happen only in mammals, such as ruminants, which have lost the internal carotid; in these, it was said (de Castro, 1926; Sunder-Plassmann, 1930), the sinus occurs at the commencement of the occipital artery ('sinus occipitalis'). This view, however, has been disputed—de Boissezon (1942, 1944*a*, *b*), for instance, denies that there is any specialization of the occipital artery in such cases; on the contrary, he says, it is the vessels of the carotid body which are the sole source of the pressor-receptor impulses here (de Castro has, in fact, long conceded that these vessels may subserve this function, but not exclusively—'ce système sensitif exerce aussi une influence sur la régularisation de la pression sanguine, bien que non d'une manière aussi évidente que le système centripète du sinus carotidien'—1928, p. 363).

Ask-Upmark, we see, not only supports de Castro, but he has gone even further: for he seems to be the first to have observed that the occipital artery may be involved *even when the internal carotid is present*—and this irrespective of whether it is a branch of the external or (as in *Erinaceus*) of the internal carotid. However, since Ask-Upmark dealt only with the gross anatomy of the bifurcation in *Erinaceus*, a more detailed, and especially a microscopical study of the region, including the

carotid body, seems desirable. This has been the particular object of the present study.

Ask-Upmark did not notice a carotid body in *Erinaceus*—yet it had actually been found long before (Schaper, 1892, p. 290), and had also just been described by Watzka (1934). In his studies of the comparative histology of the carotid body, Watzka (1934, 1943) has given the salient features of its histology in *Erinaceus*, although he omits to say whether it is of the compact (as in the cat, Kohn, 1900; cat, sheep, deer, gopher, and seal, Watzka, 1943; dog, de Boissezon, 1942; ox, Ostermann, 1952) or the disseminated ('dispersed') type (as in the rabbit, Kohn, 1900; guinea-pig, de Boissezon, 1942; pig, Watzka, 1943; and the horse, Watzka, 1943, in which nevertheless de Boissezon, 1942, says it is generally compact). From his figures, however, the organ seems to be intermediate or 'lobular' in form (as seen typically in *Macacus rhesus*, Kohn, 1900), for the cell groups ('lobules'), although distinctly separated by septa, are more closely packed than in the disseminated type, where they may be very wide apart indeed. However, it is still not clear what these differences mean, or whether a classification of the organ based on the relative amounts of stroma is at all a rational or useful one. In some mammals lobulation increases with age—for instance, the carotid body is very compact in the calf, but in the adult becomes less parenchymatous and thus more nodular (Ostermann, 1952); and in man, also, connective tissue is said to increase with age (Schaper, 1892), eventually leading to a sclerotic appearance of the whole organ (Paunz, 1923; Martínez, 1939; Watzka, 1943).

Muratori (1943), like Kohn before him (1900, pp. 96–7), has shown that the gross structure of the organ determines its vascular pattern, for in 'lobulated' or 'disseminated' forms (as in the rabbit), the capillary nets of the individual lobules remain largely separated, producing discrete vascular areas ('Gefässinselchen' of Schaper, 1892) corresponding to the 'glomeruli' described by Arnold (1865) in man; in the compact form (as in many domestic mammals—cat, dog, mouse and guinea-pig), the capillary plexus, on the contrary, is not broken up in this way, but is uniformly dense throughout the organ. Although these differences may be very striking, Muratori doubts whether they have any functional significance. Moreover, even where the carotid body is compact, as in the dog, 'où les vaisseaux et le tissu conjonctif prédominent sur le tissue glandulaire, les sections des vaisseaux entortillés, coupés en tous sens sont très apparentes et le corpuscule se présente surtout comme un glomus vasculaire' (de Boissezon, 1943, p. 138).

The hedgehog, it seems, is one of those mammals (like the horse, rat, goat, deer, mole, guinea-pig and gopher) whose carotid body contains no chromaffin cells at all (Watzka, 1943, p. 288). If it is true 'dass eine auffallende Übereinstimmung zwischen der Menge der chromaffinen Zellen und dem Anteil des Sympathikus an dem gemischten Nervengeflecht des Paraganglion caroticum der Säugetiere besteht' (Watzka, 1934, p. 113), then the sympathetic contribution to the carotid body of the hedgehog should be nil or negligible—'Je grösser der Anteil der markhaltigen (Hirn) Nerven ist, desto geringer scheint auch die Zahl der chromierbaren Zellen zu werden, um schliesslich in den an markhaltigen Nervenfasern reichsten und an sympathischen Fasern ärmsten Paraganglien gänzlich zu schwinden. Hierfür bieten die Karotisparaganglien von Igel und Reh, die ausschliesslich aus *nicht chromier-*

baren Zellen bestehen, überzeugende Beispiele' (Watzka, 1934, p. 112). Watzka also states (and has kindly provided me with a photograph to show it) that in the medullated nerves which preponderate around (and in) the carotid body of the hedgehog there are often (as in many other mammals) small nests of paraganglionic cells, which likewise never give a chromaffin reaction.

In contrast to those who believe that the specific cells may form a syncytial network (Meijling, 1938; Martínez, 1939, and others), Watzka is quite unequivocal—the non-chromaffin 'paraganglionic' cells in the carotid body of the hedgehog 'are undoubtedly of rounded form, and well-outlined' (1943, p. 286). He does not say whether the hedgehog shows the two types of nuclei ('dark' and 'light') which have been described in most other mammals (see Langer, 1952, on this point), nor does he mention other cell-types beyond stating that ganglion cells 'of pronounced cerebrospinal type' are particularly evident in the hedgehog, both among the 'paraganglionic' cells and in the associated nerve plexuses (Watzka, 1934, fig. 3, p. 111).

Since Watzka has not dealt at all with the topography, blood-supply or gross innervation of the carotid body in the hedgehog, I have paid special attention to these features. It seems to me that details such as these, which are so very important experimentally, are too often neglected, especially in regard to the carotid body, and more particularly its innervation.

MATERIAL AND METHODS

Numerous hedgehogs were used, some purely for exploratory dissections of the cervical region. Only those on which histological procedures were actually carried out are included here.

In five animals (H.1, H.2, H.3, H.4, H.12) the region of the bifurcation on one or other side was carefully removed for routine histological study. This material was fixed in 10% formol, embedded in paraffin and sectioned serially, generally at 10μ, either longitudinally or transversely; with the exceptions noted below, it was all stained with haematoxylin and eosin. (Some slides were subsequently restained with picrofuchsin and Weigert's elastic tissue stain, in order to clarify certain details.) In one series (H.1) the left bifurcation was reconstructed by the wax-plate method (Pl. 1, figs. 1, 2). Three animals of this group had been injected through the abdominal aorta, in two cases (H.2, H.4) with carmine-gelatine, and in one (H.12) with indian ink; of these, one bifurcation of H.2 was sectioned at 15μ, while both bifurcations of H.12 were sectioned at 50μ (the right longitudinally, the left, transversely). After sectioning, these were stained only with haematoxylin.

In two more animals, the bifurcation was fixed either in 5% potassium bichromate (H. 6) or in Zenker's solution (H. 9), particularly for chromaffin cells but also for optimum fixation of the specific cells (Kohn, 1900, p. 99). After embedding, both were sectioned at 10μ; the Zenker series was stained with van Gieson's haematoxylin-picrofuchsin stain, and the bichromate series, after examination for chromaffin cells, was stained with haematoxylin and eosin.

Finally, two animals were used purely to show the innervation of the region. In one (H. 7), both bifurcations after removal were placed in Bodian's fixative; after embedding and sectioning (the right transversely at 25μ; the left longitudinally

at 10μ), they were stained by Bodian's activated-protargol method. The transverse series was reconstructed graphically, using the Edinger apparatus (Text-fig. 1). In the other animal (H. 10) the vascular system was first of all irrigated, through the aorta, with a 4% sodium citrate solution (plus 0·02% sodium nitrite) until cleared of blood; it was then perfused with Bodian's fixative at a constant pressure of 200 mm. Hg. for 3 min., after which the neck was opened and the head placed in the same fixative for 24 hr. Both sides were sectioned longitudinally, the right (H. 10R) at 30μ, and left (H. 10L) at 10μ; both were stained as H. 7, except that an attempt was made, by varying the time of reduction, to suppress the background more effectively, following the suggestion of Glassner, Breslau & Agress (1954).

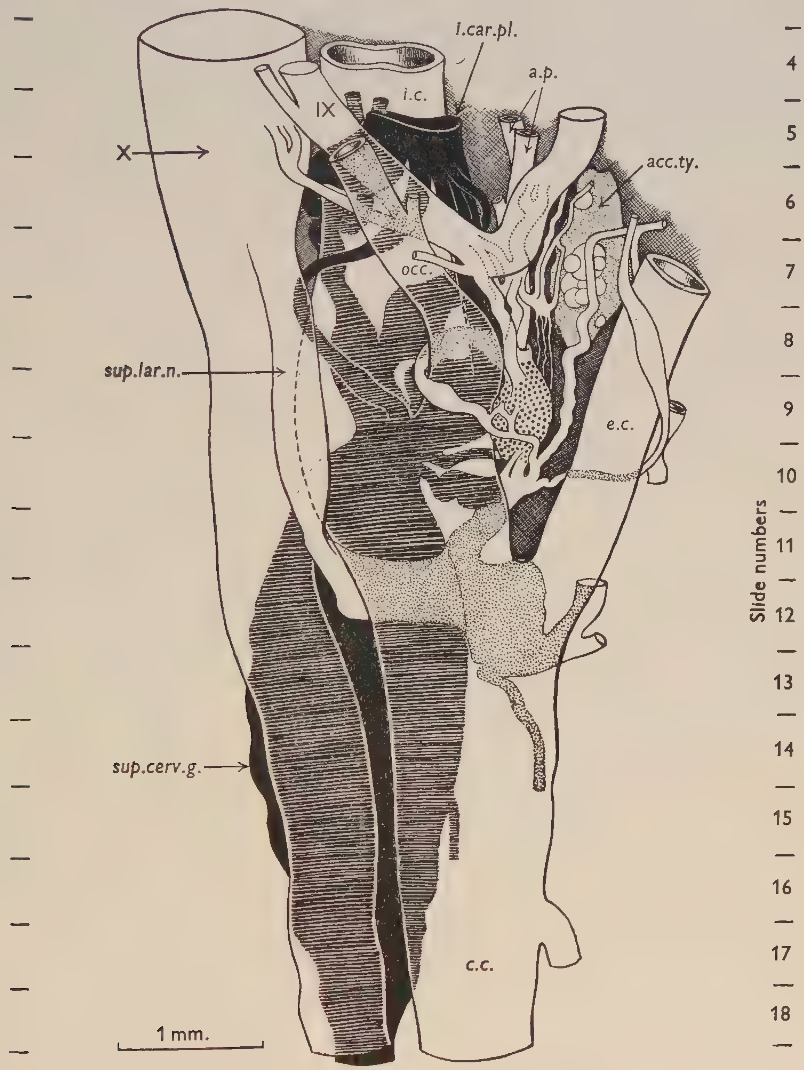
RESULTS

In *Erinaceus europaeus*, the common carotid artery divides just below the posterior belly of digastric, into a smaller ventral, external carotid and a larger dorsal trunk which, although usually called the internal carotid, is at first common to this and the occipital and ascending pharyngeal arteries. As Ask-Upmark has indicated, this common trunk is wider even than the common carotid itself (Pl. 1, figs. 1, 2); it thus forms a distinctive swelling—the carotid sinus. Shortly after its origin, this trunk gives off ventrally the occipital artery, or, more correctly, the common occipito-ascending pharyngeal artery; thereupon the dilatation of the internal carotid gradually diminishes (Text-fig. 1, Pl. 1, figs. 1, 2). The occipital artery almost immediately divides into two branches, the smaller of which, and the more ventral, is the ascending pharyngeal, which soon divides again; the larger, dorsal branch is the occipital proper—this inclines obliquely across the lateral face of the internal carotid as it passes dorsally and rostrally to the nape of the neck.

Macroscopically, the usual nerves are present (Text-fig. 1). Crossing the bifurcation superficially, but giving no branch to it, is the hypoglossal nerve. The vagus nerve runs down, as usual, behind the internal carotid; just above the bifurcation its superior laryngeal branch, leaving the lower pole of the ganglion nodosum, curves ventrally on the medial side of the vessels; there was no branch of the vagus resembling that shown by Ask-Upmark (1935, fig. 2, p. 58) on the lateral surface of the internal carotid and running directly to the bifurcation. The cervical sympathetic is dorso-medial to the internal carotid, the superior cervical ganglion lying lower than usual, just below the level of the bifurcation, to which it usually gives off two branches; these run medial to the bifurcation and, near the carotid body, they are usually joined by a small branch of the superior laryngeal nerve and all pass directly to its caudal pole.

Finally, several branches come from the glossopharyngeal nerve and its pharyngeal ramus. After crossing the internal carotid, and being joined by a communicating (? pharyngeal) branch from the vagus, the glossopharyngeal curves forwards just above the bifurcation, very close to the carotid body (Text-fig. 1; Pl. 4, fig. 18B). Here it gives off one or two short, stout intercarotid branches which follow the ascending pharyngeal artery down to the carotid body, reaching it at its superior pole. In the reconstructed specimen, additional twigs were also found to leave the pharyngeal branch of the nerve, and terminate in the carotid body (Text-fig. 1).

The carotid body itself is not easily seen, which explains, of course, why it was overlooked by Ask-Upmark. However, when one knows its position and examines the region with a dissecting microscope, a distinct swelling appears on the ventral or ventro-medial side of the ascending pharyngeal artery near its origin. This locality also appears to be the focal point for the various nerves running to the bifurcation (Text-fig. 1).



Text-fig. 1. Graphic reconstruction of the right carotid bifurcation of the hedgehog (H. 7) seen from the lateral side. An attempt has been made to indicate the gross innervation of the region as completely as possible. The following convention has been used: the sympathetic is in black, but white-lined where the ganglion or its branches are obscured (however, near the carotid body the two sympathetic branches are left white); the vagus and glossopharyngeal nerves and their branches are white, but dotted where they are obscured. The carotid body is indicated by very coarse dots and the accessory thymus by very fine dots, except for the cysts which are left clear.

Histological results

The gross features of the bifurcation, as seen by dissection, are confirmed on histological study, particularly after reconstruction (Text-fig. 1). In one specimen, a peculiar muscular trabecula was seen to cross the lumen of the common carotid just below the bifurcation, straddling the opening of the external carotid (Pl. 1, figs. 3, 4). This unusual feature is probably a persistent 'Inselbildung'. Although 'Inselbildungen' occur quite commonly, during development, in the aortic arches (Tandler, 1902; Vitums, 1951) and other arteries (Evans, 1912), it is apparently very rare for them to persist. When they do (except for a quite unique case at the carotid bifurcation, described by Adachi, 1928), it is usually due to the passage through the vessel of a nerve (Backman, 1909). Here, however, nothing passed through—the trabecula consisted simply of smooth muscle. In yet another specimen, a small accessory thymic nodule containing several cysts of varying size was observed just above the carotid body (Text-fig. 1).

The carotid sinus

Whereas in most mammals which show a swelling at the commencement of the internal carotid, the vessel wall is much thinner here than it is above and below the sinus, this is certainly not so in *Erinaceus*—on the contrary, *the wall of the internal carotid is thicker here than it is higher up, and is even thicker than that of the common carotid below* (Pl. 2, fig. 8; Pl. 4, fig. 18). This thickening involves all three coats, including the adventitia; there is, however, no dramatic increase in, or reduction of, any particular constituent as is usual in other mammals, where the elastic tissue is generally markedly increased; here, the elasticity is not excessively greater than that of the common carotid (Pl. 2, figs. 9, 10). The vessel here is quite muscular, although certainly less so than the much smaller external carotid. However, in its ventral part the vessel-wall does show a marked thinning; but this belongs strictly to the occipital artery, and not to the internal carotid. It disappears as soon as the occipital is given off (Pl. 4, fig. 18).

Both the occipital and ascending pharyngeal arteries remain very thin-walled for a certain distance beyond their origins, particularly as regards the media; the sparsity of muscular tissue here is very noticeable in comparison with the structure further on (Pl. 4, fig. 18). The thinning is quite uniform, i.e. it is no more marked on those surfaces which are adjacent to the carotid body than elsewhere. There is no obvious dilation of either the occipital or ascending pharyngeal artery, even in injected and perfused specimens (Pl. 4, fig. 18).

There is an exceedingly dense innervation around the common origin of the occipital-ascending pharyngeal trunk and the adjacent surface of the internal carotid, particularly below the actual origin of the occipital. Here a profusion of axis cylinders passes into an extensive, well-vascularized adventitial zone (Pl. 3, figs. 11, 13, 14; Pl. 4, fig. 19) in which they ramify freely (Pl. 3, figs. 14-17), and which presents the same large, swollen, irregular nuclei which are seen in similar zones ('receptor fields') in those other mammals in which they have been carefully studied (Sunder-Plassmann, 1930; Meijling, 1938; de Castro, 1940) (Pl. 3, fig. 12). Besides the fibres and endings at the origin of the occipital-ascending pharyngeal trunk,

others extend beyond the thin-walled part of the vessel, into relation with the much thicker wall of the internal carotid proper. However, the galaxy of fibres and endings fades as we pass around the belly of the sinus, and they are much sparser on the convex wall opposite the origin of the occipital trunk; the heavily innervated zone does not form an annular belt around the internal carotid as in other mammals. Thus, in spite of the fact that the occipital artery arises here from the internal carotid and not from the external carotid as it usually does in many other mammals, the innervation about the internal carotid proper, rather than being increased thereby, is, if anything, less than usual.

It is very difficult, indeed almost impossible, to determine the exact course of these fibres except that many of them, being medullated, must be of glosso-pharyngeal origin. Most of them obviously reach the region either by traversing the carotid body or by skirting round it in the 'periglandular plexus'. No substantial nerves appear to go directly to the vessel walls, so we cannot speak here of a 'sinus nerve' in the sense in which it is often used, i.e. to refer to a nerve which can be identified and separated from those nerves ensheathing, and terminating in, the carotid body—this would appear to be impossible in the hedgehog. It is interesting to note that there is some evidence of an ancillary (efferent) sympathetic innervation to the carotid sinus (Palme, 1945; Kezdi, 1954). Although it is impossible to be certain, it is not at all improbable that this could also obtain in *Erinaceus*.

The carotid body

The carotid body in *Erinaceus* is a small, rather irregular, kidney-shaped nodule, about 1 mm. long, on the ventro-medial aspect of the ascending pharyngeal artery, or the common trunk below this (Pl. 1, fig. 1; Pl. 2, fig. 8B; Pl. 4, fig. 18C). It is tucked in between the ascending pharyngeal and the external carotid arteries, although much more intimately related to the former, being as it were moulded on to it, and closely attached to its adventitia (Text-fig. 1; Pl. 5, fig. 22). It is distinctly lobulated (Pl. 1, fig. 5; Pl. 5, fig. 22), being broken up by comparatively narrow septa into groups of cells ('lobules'; 'Secundärknötchen' of Schaper, 1892) arranged around a hilar mass of connective tissue ('noyau fibreux' of de Boissezon, 1943), which fills the concavity of the organ; into this, usually nearer the lower pole of the organ, passes the artery of supply. This small vessel (which may be reinforced by additional branches—Pl. 5, fig. 22) comes from the ascending pharyngeal artery, or from the ventral wall of the main occipital trunk below this (Pl. 4, fig. 21); after branching to supply the carotid body, it may continue rostrally away from this region. In the carotid body, the artery breaks up rapidly, its main branches running radially to the lobules; some, however, pass right through and, emerging at the periphery (Pl. 1, fig. 5), supply adjacent structures, including, most likely, the vascular 'receptor fields' in the adventitia of the neighbouring vessels. The lobular arterioles give rise to complicated intralobular plexuses of sinusoidal capillaries (Pl. 6, figs. 27, 28), which drain peripherally into venules; leaving the lobules these run by way of the septa into small veins on the surface of the gland, which in turn join a large tributary of the internal jugular running downwards alongside the carotid body (Pl. 1, fig. 5; Pl. 4, figs. 20, 21). I agree with de Boissezon (1944*b*)

that multiple veins leave the organ; there is no 'venous pole' as Princeteau (1899) has described in man.

The structure of the 'lobules' is very characteristic, and quite different from the conventional descriptions. As seen in well-perfused Bodian preparations each lobule is, in fact, a miniature carotid body. It has a highly vascular connective-tissue core, surrounded by an almost continuous investment of small compact groups ('cell-nests') of epithelioid cells (Pl. 5, figs. 25, 26); in injected preparations these may look almost like an epithelial layer covering the lobule (Pl. 6, fig. 27). In the cell-nests all the cells are alike; no dark cells were evident here, nor was any other type of 'glomus' cell seen (Schwann cell nuclei, however, appear here with the nerve fibres). All the cells are discrete entities—they are well-outlined (Pl. 6, figs. 31, 32) and show no evidence of stellate form or syncytial connexion with their neighbours. Their nerve supply reaches them mainly from the periphery of the lobule, where the rich interlobular plexus of medullated and non-medullated fibres forms an investment to it (Pl. 5, fig. 25). On the contrary, the arteriole supplying the lobule plunges right into its interior through a kind of hilus and, there, immediately breaks up into a network of very tortuous sinusoidal capillaries, lying for the most part in a delicate reticular stroma which forms the central core to the lobule (Pl. 5, figs. 25, 26) and which is entirely devoid of specific 'glomus' cells. The peripheral parts of this convoluted mesh of sinusoids are directly related to, and even indent, the inner aspects of the cell-nests. Whether, however, every cell makes direct contact with a sinusoidal capillary, as de Castro claims, was not certain, but the number of cells in each cell-nest is so small that it is not all unlikely. Because the sinusoidal capillary plexus lies central to the cell-nests we might expect, in accordance with Muratori's thesis, that the vascular picture would be one of capillary 'glomeruli' ('Gefässinselchen' of Schaper, 1892) as in the rabbit and man. That this is so, is very evident from vascular injections of the gland (Pl. 6, figs. 27, 28), even though the filling of the lobules here was rather poor and incomplete. Arnold's comparison of the vascular picture in cases such as this, with that of the renal glomerulus, is not out of place; for the tortuosity of the sinusoidal capillaries gives them more than a superficial resemblance to those of the renal glomeruli, particularly of the foetal kidney where the visceral layer of Bowman's capsule forms a comparable epithelial investment over the capillaries (of course, as Kohn has emphasized, the capillaries do not form an arterial rete as in the kidney).

In the central stroma, besides endothelial nuclei (but in marked contrast to them) there are rather voluminous nuclei of varying form, which generally have so intimate a relation to the walls of the sinusoidal capillaries, that often they might easily be taken for lining cells themselves (Pl. 6, figs. 33, 34). In every respect (except that no nerve terminals were traced to them) these cells appear to resemble those described and figured by de Kock (1951, 1954) as 'glomus cells, type II'; but they are also not unlike 'Gefässwandzellen' (Schaper, 1892; Kohn, 1900), particularly in their tendency to remain diffuse and the fact that they may occur outside the lobules. Whatever the true nature of these cells, I am at least sure that no cells corresponding to de Kock's second receptor cell are present in the actual cell-nests; so that the hedgehog may prove a very suitable animal in which to verify the presence of such cells. In addition to those described, there are also ordinary extra-

vascular reticular cells which may well represent the syncytial 'interstitial' cell of de Kock (1954). Mast cells, which have been described by Marchand (1891) and Paunz (1923), and which Langer (1952) says are unusually numerous in man, were not obvious here. Nerve fibres are present, but not common, in the central core; where they occur Schwann cells accompany them, and thus add to the nuclear population there.

As stated, the interlobular septa of the organ are particularly rich in medullated and non-medullated nerve fibres; the former lose their medullary sheaths only as they pass into the lobules where they come into direct relation with the specific cells. Cell-nests at the periphery of the organ may be supplied directly by fibres from the periglandular plexus. No convincing endings were seen, so that it is not possible to say how precisely the fibres terminate in relation with the cells.

In addition to the specific cells, ganglion cells, isolated or in groups, are, as Watzka has emphasized, very frequent here, particularly in the nerve fasciculi outside the carotid body (Pl. 6, fig. 29), but also inside it, and occasionally even alongside parenchymal cells (Pl. 5, fig. 25; Pl. 6, fig. 31). The ganglion cells and microganglia outside the carotid body were more commonly associated with the sympathetic nerves going to the organ than with the glossopharyngeal branches although they were also occasionally seen there; furthermore, they are very similar to those of the adjacent superior cervical ganglion (Pl. 6, fig. 30). Conversely, as Watzka has also shown, cells identical with those of the carotid body may occur in small groups in the nerve trunks outside the organ.

The nerve supply of the carotid body comes from two main sources: the glossopharyngeal nerve, and the superior cervical ganglion of the sympathetic chain (Text-fig. 1). There is also a small contribution from the vagus, by way of twigs from the superior laryngeal nerve which may join the sympathetic branches; although these enter the carotid body near its inferior pole I do not know whether the fibres terminate in it or pass to the adjacent vessel wall (Pl. 5, fig. 22). The hypoglossal nerve gives no fibres either to the carotid body or the carotid sinus in the hedgehog. The *sympathetic* fibres leave the superior cervical ganglion (generally near its upper pole) by two very short, thick trunks composed exclusively of non-medullated fibres. These run directly ventrally, to the carotid body (Pl. 4, figs. 20, 21). The lower branch usually joins that from the superior laryngeal (Pl. 4, fig. 20) and runs with it to the inferior pole of the organ; it was not clear what the fate of these fibres is, although many probably by-pass the carotid body to enter the external carotid plexus (Pl. 4, fig. 20). This branch in particular showed a group of ganglion cells close by the carotid body (Pl. 6, fig. 29). The upper branch runs directly to the medial aspect of the organ (Pl. 4, fig. 21), and after sending fibres into it, turns rostrally on its surface and thus skirting it, rejoins the main internal carotid (sympathetic) trunk at the upper pole of the organ (Pl. 5, fig. 22); here further fibres are either given off to, or received from, the carotid body (Pl. 5, figs. 23, 24). The medullated *glossopharyngeal* fibres arise by one or two branches which, after a very short course, reach and enter the upper pole of the carotid body; as stated, other delicate twigs may leave the pharyngeal branch of IX, and run down together to reach the ventro-medial surface of the carotid body, which they enter (Text-fig. 1). The majority of the glossopharyngeal fibres undoubtedly terminate in the gland, although as suggested above, others probably skirt it, or

traverse it, to reach the specialized arterial region at the origin of the occipital and ascending pharyngeal arteries.

Clearly, then, there is no one 'nervous pole' in de Boissezon's sense (1942), for the glossopharyngeal branches enter the carotid body near its cranial pole, while the sympathetic and vagal fibres reach it caudally. Furthermore, although there is some anastomosis between the nerves as they pass to the organ (as between the lower sympathetic and superior laryngeal branches), there is no intercarotid plexus in the classical sense (see Boyd, 1937), for the branches contributed by the separate nerves largely retain their independence until at or near the gland, where they form intricate and dense periglandular and intraglandular plexuses; it then becomes impossible to determine the sources of the fibres ramifying in them.

DISCUSSION

My findings in regard to the carotid body in the hedgehog largely confirm the descriptions already given by Watzka. However, I am by no means sure that the carotid body of the hedgehog, because it has no chromaffin cells has *necessarily* no sympathetic innervation, for beyond doubt it receives fibres from the superior cervical ganglion and, what is more, numbers of these can certainly be followed into the organ; but since it was not possible to trace them to the specific cells in the lobules, it cannot be denied that they may merely traverse it. De Castro (1928, 1940) has suggested that if such fibres do end in the carotid body, they must be effector fibres to the muscular arterioles of the organ, with no sensory function at all. It is, however, impossible, by simple histological examination, to come to any decision on this point; this would require experimental analysis such as de Castro has carried out on other mammals.

I find, like Watzka, that near the carotid body in the hedgehog there are an unusually large number of ganglion cells and microganglia. Where they could be assigned to definite nerve trunks, these ganglion cells appear to me to be more common in relation with sympathetic nerves than with the branches of the glossopharyngeal—thus the view that they are principally 'cerebrospinal' neurones (Muratori, 1932, 1933, 1934; Watzka, 1943) may not apply to the hedgehog; here there is a strong possibility that they may be predominantly orthosympathetic, as de Castro (1926, 1928) believed them to be in the mammals he studied. The occurrence of ganglion cells and microganglia in such numbers, while quite common in some mammals (in the horse, for instance, de Boissezon, 1942, says: 'Au niveau de l'insertion du corpuscule carotidien...se voient un très grand nombre de microganglions qui forment, en ce point, un véritable centre nerveux', p. 51), is however in contrast to other mammals such as *Trichosurus vulpecula*, where although there is apparently a large sympathetic contribution (at least to the intercarotid plexus), ganglion cells are quite infrequent (Adams, 1955). My findings, moreover, do not accord with those of Ask-Upmark (1935) as to the vagal contribution to the innervation of this region. Instead of the obvious branch he describes and figures, arising from the vagus, all I could find were comparatively few fibres going to the carotid body from the superior laryngeal nerve; there were no branches of the vagus going directly to the 'sinus' region.

The blood supply of the carotid body of the hedgehog, in so far as the capillary

bed is broken up into lobular 'glomeruli', is very like that of man (Arnold, 1865; Paunz, 1923), the rabbit (Muratori, 1943; Chungcharoen, Daly & Schweitzer, 1952 *b*), and the ruminants (de Boissezon, 1942, 1943); however, in the hedgehog, the sinusoidal capillaries of the 'glomeruli' do not pervade the entire lobule but are confined to its central connective-tissue core, i.e. they are covered over by the cell-nests of the lobule. In addition to the vessels which supply the parenchyma of the organ, there are others which pass right through to be distributed outside it ('artères de dérivation'). Such vessels, which have been frequently described, and not only in the mammals mentioned above, may form important physiological by-passes linking the carotid bifurcation with the cerebral circulation, as Chungcharoen, Daly, Neil & Schweitzer (1952 *a*) have pointed out. I am not able to say whether or not any of these vessels, as de Boissezon suggests (1943, p. 138), pass 'directement, dans les larges veines qui entourent l'organe' (thus forming a direct arterio-venous anastomosis), or whether, as one certainly did, they merely continue on, as arteries, in the intercarotid triangle. Muratori (1943) has noted in mammals, and Chowdhary (1953) in the fowl, that some of the arterioles leaving the organ supply the walls of the neighbouring vessels. In the hedgehog, also, the adventitial zones in the sinus receptor regions are particularly vascular; but I am not certain whether these regions are actually supplied by way of the carotid body. It is, nevertheless, a fact worth bearing in mind, because of its obvious physiological implications.

I have been particularly fortunate in being able to clarify the detailed structure of the lobules which make up the carotid body in the hedgehog. This has been possible only in animals in which perfusion-fixation has been carried out, under pressure. This effectively dilates the sinusoidal capillaries, and apparently also produces some oedema of the connective-tissue core of the lobule. Thus artificially distended, the true structure of the lobule becomes apparent, whereas in routine preparations, with immersion fixation, the intralobular vessels are collapsed; the lobules are then much more compact, so that it becomes difficult, if not impossible, to determine their true constitution. The lobules in the hedgehog, it turns out, are not solid balls of epithelioid cells, as usually described, with the capillaries ramifying between them, but consist of a central core of loose vascular connective tissue around which the specific ('chief') cells are disposed in small clusters (cell-nests). Thus the sinusoidal capillaries lie central to the cells, whereas the nerves, paid off from perilobular plexuses, reach the cells from the periphery of the lobule. Moreover, the cells in the hedgehog do not occupy the meshes of a capillary plexus, as Schaper (1900, p. 300) thought they do in man, and as they are generally believed to do in other mammals.

The fact that in ordinary preparations this feature is not very evident suggests that, in such cases, cells which normally form part of the central stroma might easily be misinterpreted as belonging to the cell-nests, and be considered as a second cell-type. Thus the syncytial cells which have been described (in addition to the 'chief' cells) by de Boissezon (1942) and de Kock (1954) may merely represent anastomosing reticular cells of the stroma of the lobule. At any rate, in the hedgehog, no such syncytial cells are present in the cell-nests; nor, as I have explained, could I identify de Kock's (1951, 1954) second type of glomus cell there, although it may well be represented in the 'core' of the lobule.

The arrangement of the sinusoidal capillaries, and their relations to the specific cells in the lobules of the carotid body of the hedgehog, are quite unlike those seen in arterio-venous anastomoses, where the epithelioid muscle cells form a much more intimate investment of the vessels concerned. The minute structure of the carotid body of the hedgehog has, therefore, nothing in common with that of arterio-venous anastomoses, as some have claimed (Schumacher, 1938; Goormaghtigh & Pannier, 1939, who however do not regard the specific—'glomus' or 'paraganglionic'—cells of the lobules as myoepithelial, but as an 'annexe paraganglionnaire...implantée sur les anastomoses artério-veineuses qui constituent le glomus,...au niveau du segment préveineux où leur paroi n'est pas plus épaisse que celle d'un capillaire', p. 522). Hollinshead (1942), moreover, has conclusively shown that, in man and the cat, the lobular circulation is certainly not part of an arterio-venous anastomosis. Da Costa (1944) and de Castro (1951), who have also described arterio-venous anastomoses here, make it quite clear that these 'anastomoses artério-veineuses, proprement dites, (sont) situées la plupart dans le conjonctif environnant, à l'entrée même de l'organe' (da Costa, cited by de Castro, 1951, p. 19); they are not therefore an integral feature of the circulation *through* the lobule, as Goormaghtigh & Pannier held, but are rather physiological by-passes, shunting the blood *away from* the lobules of the organ (de Boissezon, 1943; de Castro, 1951).

It is obvious, from the fact that other structures in the region (such as the hibernating gland and the various ganglia) were well-injected (Pl. 1, figs. 6, 7), that there must have been some obstacle to the filling of the vascular bed of the carotid body in the hedgehog. The results were fickle and unpredictable; some lobules were well filled, others not at all, while in many, although a certain amount of the injection mass had entered the lobules, the filling of the intralobular plexus was obviously incomplete. These defects suggest that there is some—possibly a physiological—barrier to the ready filling of the intralobular vessels; this may be due to the activity of the prelobular arterioles, which are distinctly muscular, although in the hedgehog there does not appear to be any structural specialization of these vessels like the epithelioid muscle cells described by Goormaghtigh & Pannier in the cat, or 'les épaississements' of these arteries found by de Boissezon (1943) in the horse. De Boissezon (1943, p. 138) claims that 'les artérioles portant ces épaississements distribuent le sang aux lobes glandulaires'; and again (1942, p. 123) that 'les choses paraissent être disposées de telle sorte qu'une élévation de la pression sanguine est nécessaire pour que le territoire capillaire du corpuscule soit complètement irrigué par le sang artériel'. Although I have not found these thickenings, it is nevertheless possible that the muscular prelobular arterioles in the hedgehog may be capable of performing a function like that which de Boissezon ascribes to them. If so, it is quite likely that arterio-venous anastomoses will also be present, although I have not actually identified them.

It now appears that the occipital, or the ascending pharyngeal, or both arteries, as well as the vessels which supply the carotid body (whatever their source), are all to be considered an integral part of the carotid vasosensory apparatus. As we have seen, de Castro (1926, 1928) and Sunder-Plassmann (1930), from the very first, had considered the occipital artery to be of importance where the internal carotid is absent (ruminants particularly); at that time, however, although de Castro recog-

nized the sensory innervation of the 'glomus' vessels, neither he nor Sunder-Plassmann suspected that the occipital artery might be involved even when the internal carotid is present. Ask-Upmark realized, too, that the occipital artery was, as he put it (pp. 96, 97), 'in a privileged position'; for when the internal carotid regresses (dog, and certain other carnivora) or disappears (ruminants; some carnivores, e.g. cat) either the occipital artery (*Equus*, *Bos*, *Ovis*, *Capra*, *Canis*—Ask-Upmark, 1935, p. 122) or the ascending pharyngeal (cat, pig—Daniel, Dawes & Prichard, 1953, p. 202) may then participate in the cerebral circulation—in some cases (as in the replacement of the internal carotid by the ascending pharyngeal artery in the pig—Daniel, Dawes & Pritchard, 1953) the contribution may be very significant. Although Ask-Upmark, like de Castro, regarded the occipital artery as entirely subordinate to the internal carotid, nevertheless he was aware of its general significance, and deserves considerable credit for drawing attention to it, as well as for indicating how remarkably little is known of its development, particularly as concerns its relationship to the third aortic arch, which has generally been considered the development locus of the carotid sinus (Schäfer, 1877; Schwalbe, 1878; Sunder-Plassmann, 1930). I have, however, discussed this aspect of the problem fully elsewhere (Adams, 1956).

In 1940 de Castro published a most important paper in which, after acknowledging that he had previously underestimated the full extent of the pressor-receptor zone, he went on to show that, in the dog and the cat, besides the endings in the carotid sinus proper and those he had earlier described in the walls of the 'glomic' vessels (1926, 1928), there were still other endings in abundance in the wall of the occipital artery up to and including the origin of its branch (or branches) to the carotid body, i.e. to the very part of the occipital artery to which Hovelacque, Maes, Binet & Gayet (1930) had earlier traced fibres of the 'sinus nerve'. Furthermore, as de Castro showed, this section of the occipital artery presents a thinning of the media, similar to that around the 'belly' of the carotid sinus.

Shortly afterwards (but clearly unaware of these more recent conclusions of de Castro) de Boissezon (1942) stressed the special significance of the vessels supplying the carotid body (whatever their source) and discounted almost entirely that of the carotid (or occipital) sinus itself. He thus reverted to a modification of Drüner's original concept (1925), namely, that the pressor-receptor mechanism is largely or entirely subserved by the vessels supplying the carotid body, and that the internal carotid plays a negligible part, or no part at all, in this: its innervation, de Boissezon says, is in fact no greater than that of comparable vessels elsewhere in the vascular system. Since, in the great majority of mammals, the vessels supplying the carotid body appear to be derived directly or indirectly from the occipital or ascending pharyngeal arteries, de Boissezon would probably include these with the 'glomic' arteries proper, even although they are not exclusively concerned with supplying the carotid body. Indeed, as he pointed out, and as is now well-known (Muratori, 1943; Chungcharoen *et al.* 1952*b*), even the artery supplying the carotid body may often supply other tissues as well; also, as we have seen, branches often pass right through the organ to supply surrounding tissues (de Boissezon, 1942; Muratori, 1943).

Thus, both de Castro and de Boissezon would probably agree in assigning to the occipital artery (where it supplies the carotid body) and its 'glomic' branches,

a significant part in the pressor-receptor mechanism; but whereas de Castro and the majority of workers would extend this function also (and pre-eminently) to the carotid sinus proper in those mammals which have an internal carotid artery, de Boissezon on the other hand prefers to restrict it largely, or entirely, to the vessels directly concerned in supplying the carotid body and to regard the internal carotid as essentially a mechanical 'regulator' of the circulation, with merely an 'hydraulic' function.

The arrangements in the hedgehog have a considerable bearing on this controversy, since although here there is apparently a large carotid sinus, this does not however show the same specialization of its wall which characterizes the carotid sinus of other mammals. Not only is the tunica media not reduced at all in thickness or muscularity, but the wall of the artery here is, in fact, considerably thicker than that of the internal carotid above the sinus and of the common carotid below it; although there is no comparable increase in elasticity. By contrast, both the occipital and ascending pharyngeal arteries and the short common trunk from which they arise have very thin walls, much thinner and more fibrous than they are farther on. Furthermore, there is a great concentration of nerve endings in the specialized adventitia of these vessels at their origin from the internal carotid. These endings, moreover, encroach on the adventitia of the internal carotid beyond and below the origin of the occipital, the population of fibres and endings tending to fade away the farther one goes from this origin.

The hedgehog then is an example of a mammal in which, having the internal carotid and the occipital artery arising in common, it is the occipital artery and its ascending pharyngeal branch which appear not only to be the significant source of blood supply to the carotid body but also to dominate the pressor-receptor function of this zone. Indeed, the internal carotid would seem to be insufficiently specialized even to perform the function which de Boissezon has attributed to it—that of a passive mechanical 'regulator' of the cerebral circulation. Nevertheless, a part of the internal carotid, i.e. adjacent to and surrounding the origin of the occipital artery, does receive a very substantial innervation; indeed, the fact that the innervation was just as dense in this thick-walled region as it is around the adjacent thin-walled occipital trunk, seems to me to discount any consistent structural differences of the kind described by Boss & Green (1954*a*, *b*), who, moreover, ignore a very striking characteristic of these ('baroceptor') regions—i.e. the profusion of large bloated nuclei in a very vascular adventitia and in intimate association with the nerve fibres and their terminations. This is a feature which has been noticed and commented on by all who have worked on the detailed histology of this region, although, it is true, little or nothing is known of its significance.

De Castro has suggested that the occipital artery takes on a pressoreceptor function when it contributes to the cerebral circulation (1940, p. 336): 'La sensibilidad reflexógena seno-carotídea en el perro, no se constriñe solo al seno, se extiende también algo por sus inmediaciones en la carótida externa y singularmente por la arteria occipital durante su primer segmento. Esto mismo se puede hacer extensible al gato y quizás a todos aquellos animales cuya arteria occipital significa un vector importante en la irrigación sanguínea del encéfalo y del *glomus caroticum*'.

But in the hedgehog neither the occipital nor the ascending pharyngeal artery

appears to be at all concerned in supplying the brain—yet here the ascending pharyngeal particularly is the source of blood supply to the carotid body, and the occipital and ascending pharyngeal arteries are very obviously important presso-receptor sources.

Thus the possibility arises that the commencement of the occipital and ascending pharyngeal arteries might have an embryological significance which has, in fact, been hitherto unsuspected. Does this part of the occipital artery, for instance, correspond to a persisting part of the ductus caroticus? This is also a question which I have discussed more fully elsewhere (Adams, 1956).

SUMMARY

1. A persistent 'Inselbildung' has been observed at the carotid bifurcation in one hedgehog; and an accessory thymus in another.

2. The 'carotid sinus' in the hedgehog incorporates the common origin of the occipital and ascending pharyngeal arteries; here these arise from the ventral surface of the internal carotid artery. The rich nerve supply of this region is concentrated on the commencement of the occipital and ascending pharyngeal arteries in a well-vascularized cellular adventitia. It encroaches also to some extent on to the wall of the internal carotid artery, which in the hedgehog is *thicker* in the sinus region than above and below. The density of nerve-endings in the adventitia falls off as we pass away from the origin of the occipital artery. There is no special 'sinus nerve' in the hedgehog.

3. The carotid body is attached to the adventitia of the ascending pharyngeal artery from which it usually receives its blood supply. The kidney-shaped organ is lobulated, the lobules being disposed about a hilar mass of fibrous connective tissue into which the main artery runs. Each lobule is a miniature carotid body—nests of 'chief' or specific cells surround a well-vascularized, delicate connective-tissue core, in which lie convoluted sinusoids, fed by a lobular arteriole and draining into peri-lobular veins. 'Artères de dérivation', traversing the carotid body, are present, and may assist in supplying the very vascular adjacent adventitia. The vascular picture, in accordance with the lobular architecture, is 'glomerular' as in the rabbit and man.

4. There are no chromaffin cells in the carotid body of the hedgehog. Numerous ganglion cells and microganglia are present often in relation with orthosympathetic trunks which reach the caudal pole of the organ, in contrast to the branches of IX which reach its cranial pole. Some fibres of X (from the superior laryngeal nerve) join one of the two sympathetic branches.

5. The specific cells of the lobules are well-outlined; they are not syncytial. There is no other type of glomus cell in the cell-nests. In the connective-tissue core, however, in addition to endothelial nuclei of the sinusoids, reticular nuclei and Schwann nuclei, there are some voluminous, irregular nuclei which often have a peri-vascular situation. Although these resemble de Kock's 'second receptor cell', no nerve fibres were traced to them; no positive conclusion was arrived at as to their nature.

My thanks are due particularly to Mr J. G. Howard, Technical Officer of my Department, for his capable and experienced co-operation, not only in the technical procedures, but also in the photographic reproduction of the sections; for in this he

has taken endless pains to secure the most perfect results possible—these, I think, speak for themselves. Also, to Miss M. Ogilvie I wish to express my thanks for her patient and uncomplaining assistance in the preparation of the manuscript.

Finally, I wish to thank Prof. Alfred Kohn of Prague for his stimulus and encouragement, and for his help in trying to track down the source of the early reference by Schaper (1892) to the carotid body of the hedgehog; that we did not succeed, does not lessen my gratitude. Recently, however, I have found that the source is Pförtner (1869) in *Z. rat. Med.* **34**, 240–256.

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KEY TO ABBREVIATIONS

acc.ty	accessory thymus	*i.c.n.*	internal carotid nerve (sympathetic)
a.d.	'artère de dérivation'	*occ.*	occipital artery
a.p.	ascending pharyngeal artery	*s.*	sinusoidal capillary
c.b.	carotid body	*s.c.g.; sup.cerv.g.*	superior cervical ganglion
c.c.	common carotid artery	*s.l.n.; sup.lar.n.*	superior laryngeal nerve
e.c.	external carotid artery	*sy.*	sympathetic fasciculus
e.c.p.	external carotid plexus	*v.*	vein draining carotid body
gl. IX	branch of glossopharyngeal to carotid body	IX	glossopharyngeal nerve
i.c.	internal carotid artery	X	vagus nerve
i.car.pl.	internal carotid plexus of sympathetic		

EXPLANATION OF PLATES

PLATE 1

Fig. 1. H. 1. Wax plate reconstruction, seen from the lateral side, of the left carotid bifurcation. In the process of reconstruction, the projections were reversed, therefore the preparation appears as if it were from the right side.

Fig. 2. H. 1. The same, from the medial aspect. The arrow marks the level of the section in fig. 8B, Pl. 2.

Fig. 3. H. 1/24. Section of common carotid artery to show the muscular trabecula sectioned in the lumen. Haematoxylin and picrofuchsin. ×40.

Fig. 4. H. 1. The same reconstruction as fig. 1, opened up to show the muscular trabecula crossing the opening of the external carotid (*e.c.*). The origin of the occipital artery (*occ.*) from the internal carotid is also seen.

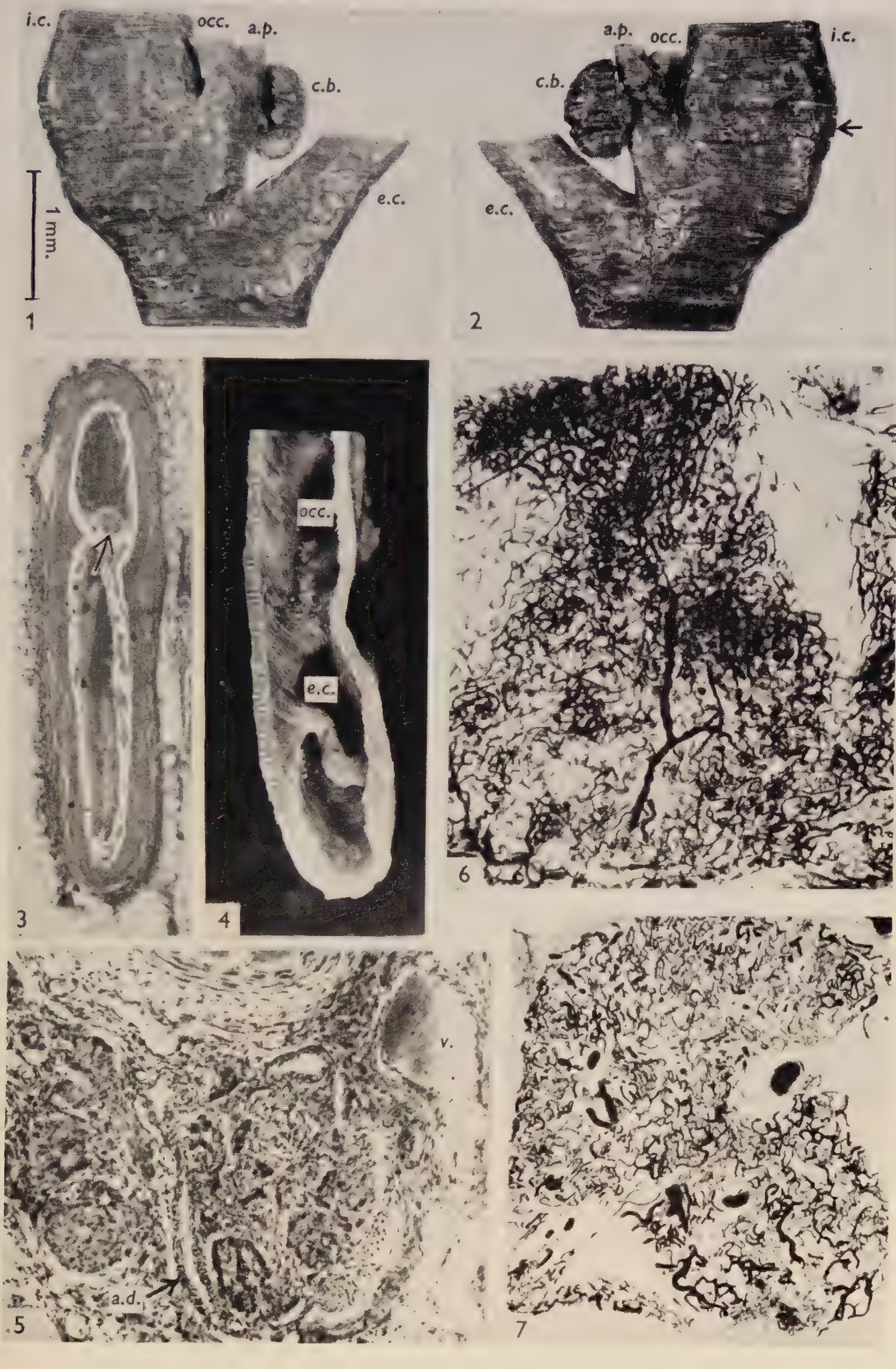
Fig. 5. H. 7/R10. Transverse section of carotid body, showing particularly a small artery ('artère de dérivation', *a.d.*) passing right through it. The lobular structure is evident. The internal carotid-occipital trunk just appears at the upper border of the figure; and a moderately large vein, which drains the organ, lies to the right. Bodian. ×100.

Fig. 6. H. 12/R4. Hibernating gland. Representative section of gland injected with indian ink. 50μ. × 156.

Fig. 7. H. 2/L5. Hibernating gland. Representative section of gland injected with carmine-gelatine. 15μ. × 156.

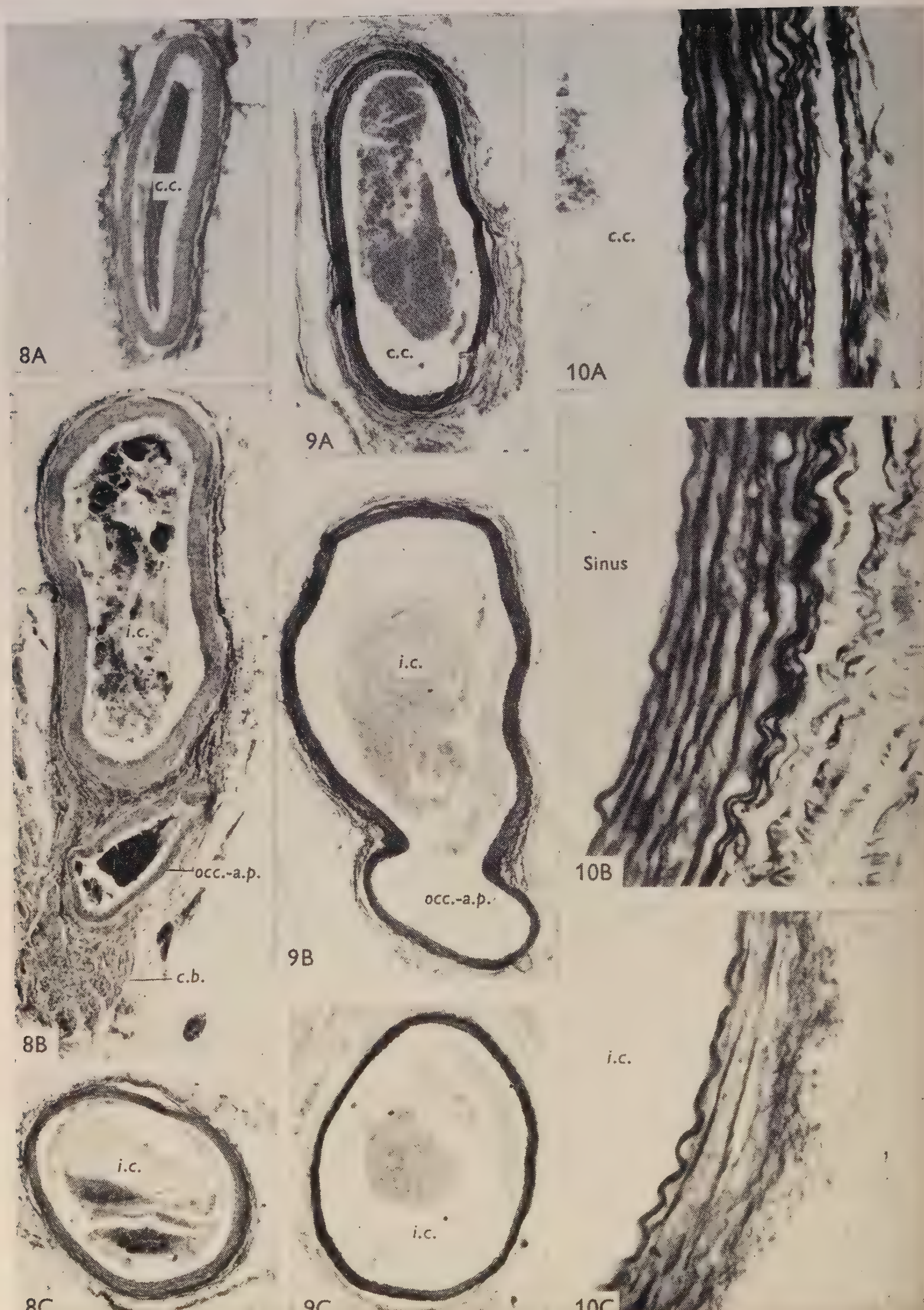
PLATE 2

Fig. 8. H. 1/31, 17, 6. Sections through: A, the common carotid artery, 3·3 mm. below the next section; B, the internal carotid just above the origin of the occipital-ascending pharyngeal artery (*occ.-a.p.*), which has the carotid body (*c.b.*) applied to its ventromedial wall; C, the

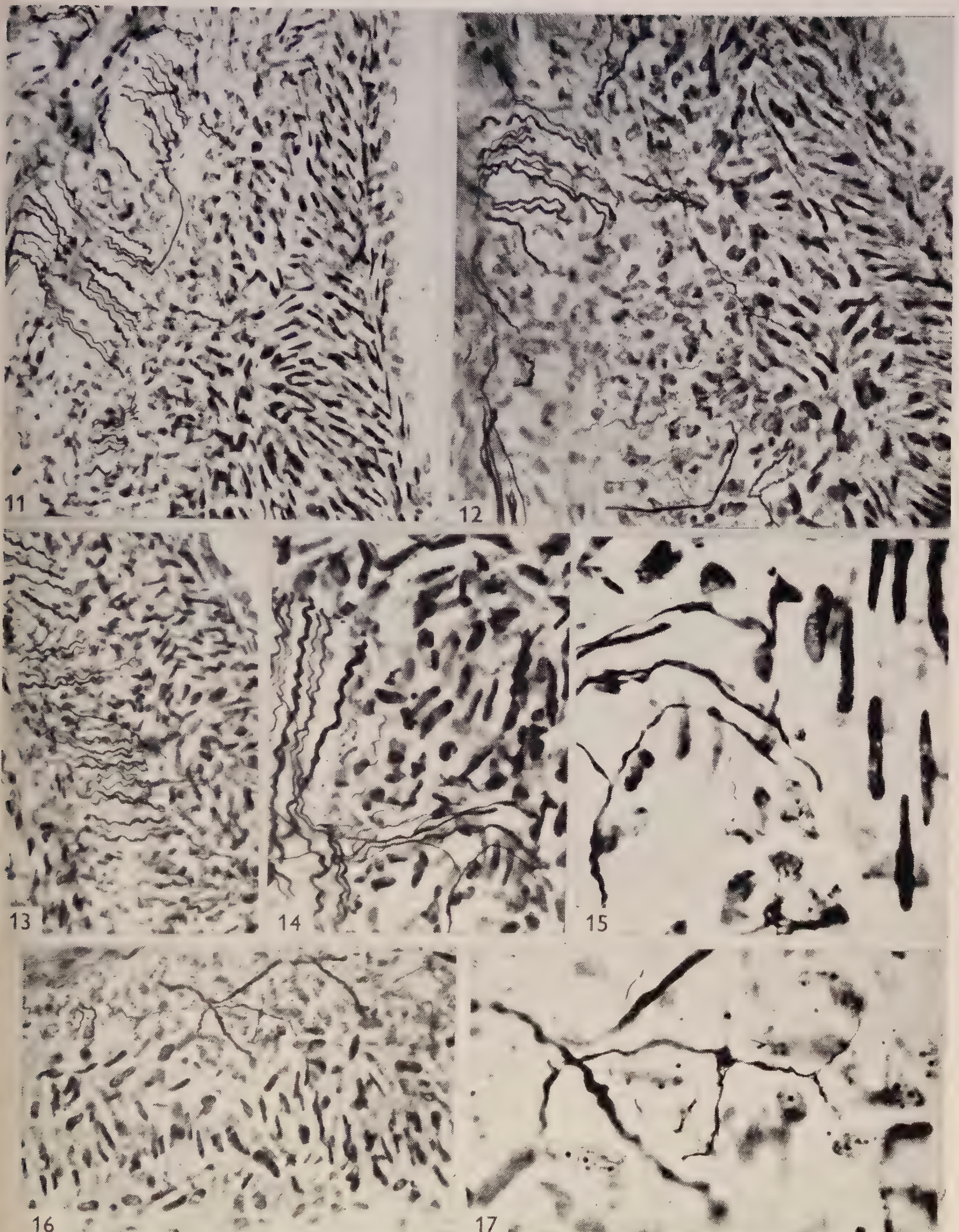


ADAMS—HEDGEHOG CAROTID SINUS COMPLEX

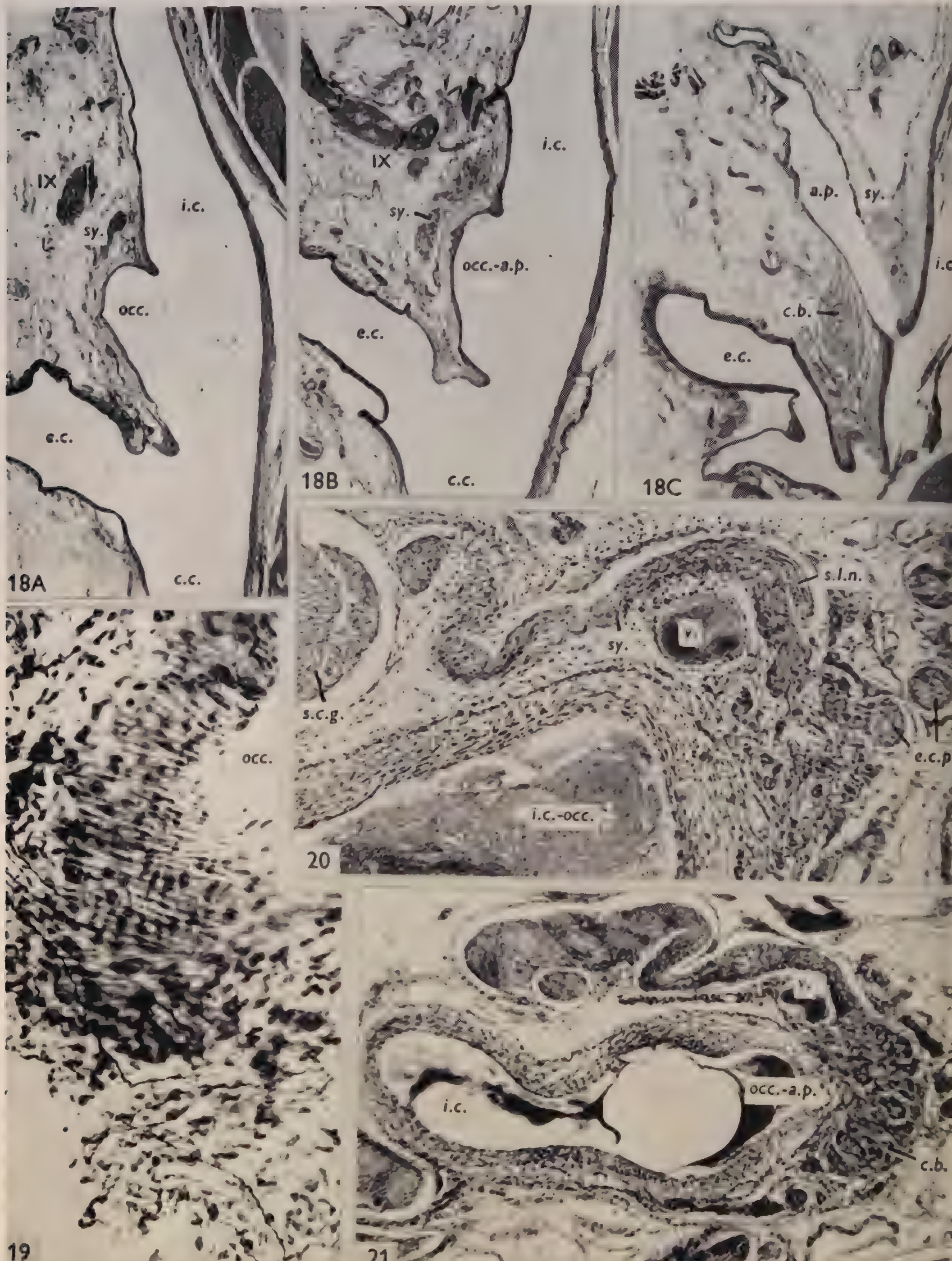
(*Facing p.* 224)



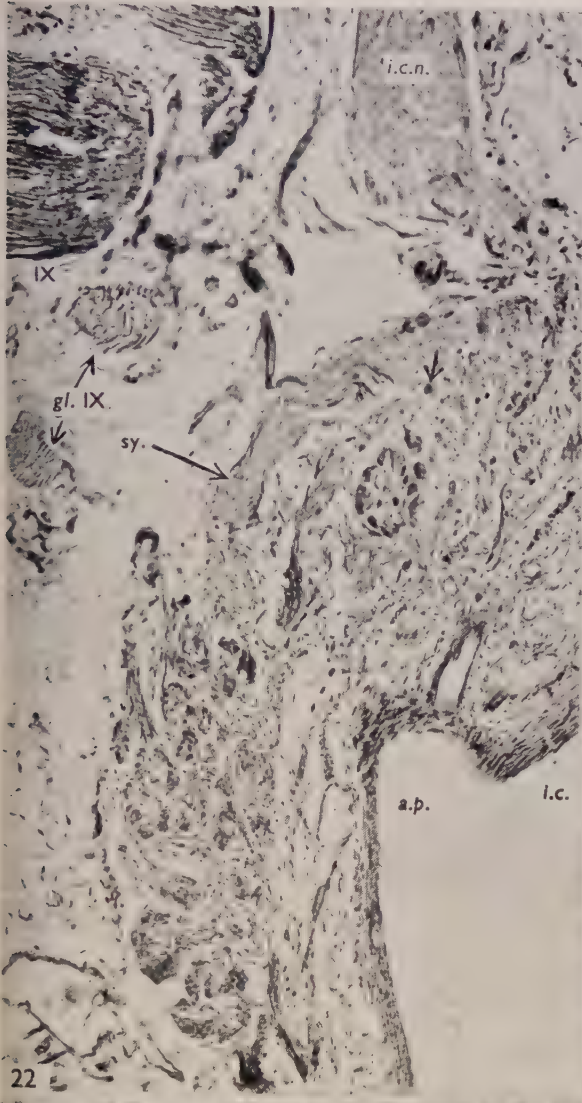
ADAMS—Hedgehog carotid sinus complex

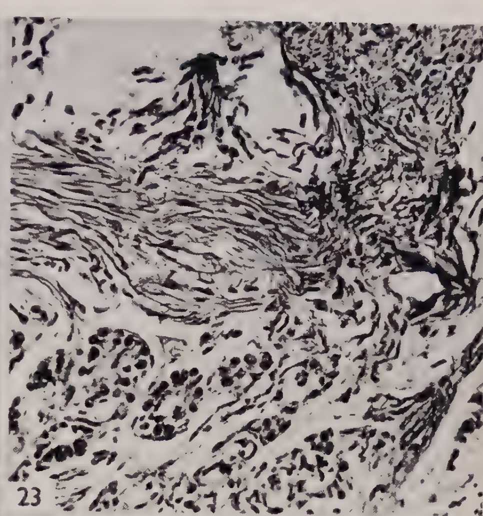


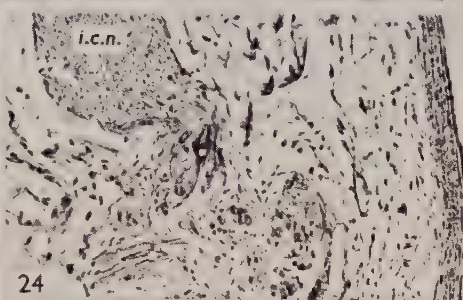
ADAMS—Hedgehog carotid sinus complex

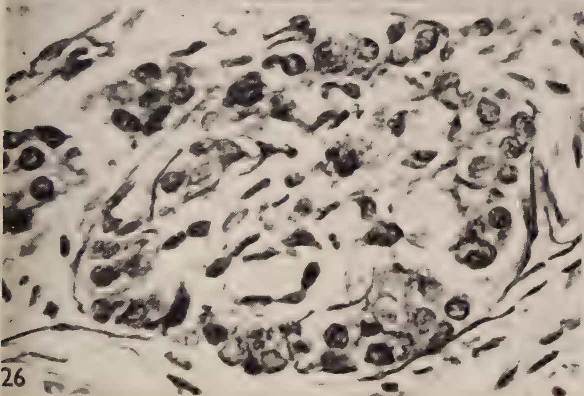


ADAMS—Hedgehog carotid sinus complex

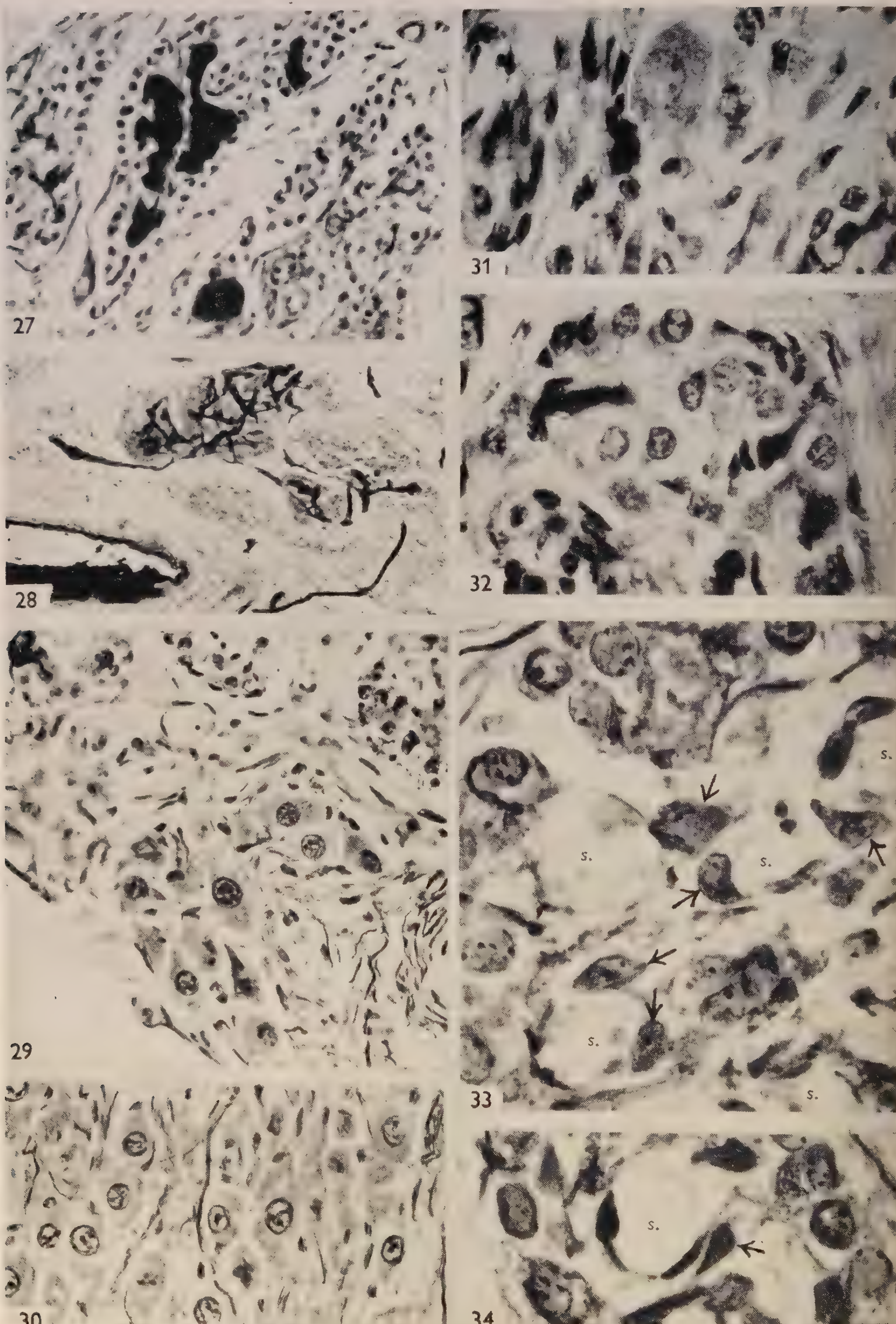








ADAMS—Hegdehog carotid sinus complex



ADAMS—Hedgehog carotid sinus complex

internal carotid, 2·6 mm. above the previous section but below the origin of the stapedial artery, i.e. before any further branch is given off. This series shows not only the marked increase in diameter at the level of the sinus, but also the increased thickness of the wall here. (Note: it is this series from which the reconstruction in figs. 1, 2, Pl. 1, was made, but the lowest section (A) is just below, and the upper (C) a little above the part reconstructed; B is approximately at the level of the arrow in fig. 2). Haematoxylin and picrofuchsin. All $\times 40$.

Fig. 9. H. 3/35, 23, 5. A comparable series in another animal to show the varying elasticity. A, is of the common carotid, 1·7 mm. below the next section; B, is through the sinus right at the origin of the occipital artery (*occ.-a.p.*); C, is of the internal carotid, 2·5 mm. above B, but again before the stapedial artery comes off. Weigert. All $\times 40$.

Fig. 10. H. 3/35, 23, 5. The same vessels showing representative parts of the wall of the vessels at a higher magnification. The lumen of the vessel is to the left. Weigert. All $\times 350$.

Plate 3

Figs. 11, 13. H. 10/L13. Sections of the wall of the internal carotid-occipital trunk, below the origin of the occipital but in the part of the wall corresponding to it, i.e. ventrally. The lumen of the vessel is to the right, i.e. dorsally. The carotid body is just out of the picture above and to the left (see Pl. 4, fig. 18C). Note the profuse innervation by medullated fibres, passing in pectinate fashion to the adventitia in which they ramify. Bodian. $\times 200$.

Fig. 12. H. 10/L14. A slightly more tangential section of the same vessel at the same level. The lumen of the common trunk is to the right, the media is obvious and outside it the very cellular adventitia ('receptor field') in which the nerve terminals spread. The bloated, irregular nuclei characteristic of these regions are very apparent; fine axons can be seen ramifying among them. Bodian. $\times 280$.

Fig. 14. H. 10/L13. A localized portion of the cellular adventitia in the same region, with (above and to the right) the media of the vessel. Bodian. $\times 370$.

Fig. 15. H. 10/L13. The lower right quadrant of the previous figure at much higher magnification. To the right, smooth muscle nuclei of the media; to the left, the 'receptor field' with terminal ramifications. Bodian. $\times 850$.

Fig. 16. H. 10/L14. Another part of the receptor region. In this figure, the lumen of the internal carotid-occipital trunk is below; above the intima is the media, followed by the cellular adventitia in which are seen the ultimate and penultimate ramifications of axis cylinders. Bodian. $\times 280$.

Fig. 17. H. 10/L14. The central part of the 'receptor field' of the previous figure shown at a higher magnification. Bodian. $\times 850$.

Plate 4

Fig. 18. H. 10. Three longitudinal sections in sequence through the bifurcation, from lateral to medial. The first two clearly show the thickening of the wall of the internal carotid at the level of the sinus, compared particularly with that above. A (L 10/1) passes through the origin of the occipital artery (*occ.*), the glossopharyngeal nerve being sectioned just superior to this; B (L10/11), also through the occipital-ascending pharyngeal origin, shows the glossopharyngeal nerve (IX) curving forwards just above the bifurcation, while ascending sympathetic trunks (*sy.*) run upwards close to the occipital artery; C (L12) cuts the ascending pharyngeal longitudinally, and thus shows clearly the thinner wall at the commencement of the artery; it also shows the carotid body, tucked in between it and external carotid artery, and partially embracing the ascending pharyngeal. Bodian. All $\times 19$.

Fig. 19. H. 10/R4. Tangential section at the origin of the occipital artery to show the 'receptor field' in this region with ramifying axons. Bodian. $\times 200$.

Fig. 20. H. 7/R10. Transverse section of the bifurcation, showing the lower sympathetic branch (*sy.*) being joined by fibres of the superior laryngeal nerve (*s.l.n.*) and reaching the lower pole of the glomus (see Text-fig. 1). Many of these fibres appear to pass into a trunk of the external carotid plexus which is forming on the right. Bodian. $\times 80$.

Fig. 21. H. 7/R9. The same series at a higher level, through the upper sympathetic branch to the carotid body. This branch reaches the body near its inferior pole. Both this nerve and the lower branch curve around a tributary of the internal jugular which drains the carotid body. The artery supplying the carotid body is just arising from the occipital part of the internal carotid-occipital trunk. Bodian. $\times 45$.

Plate 5

Fig. 22. H. 10/L11. Longitudinal section through the carotid body showing its lobular structure and close relation to the origin of the ascending pharyngeal artery (*a.p.*). At left upper corner the glossopharyngeal nerve is seen curving forwards just above the carotid body, to which it has given off a branch (*gl. IX*). Skirting the upper pole of the carotid body, in the periglandular plexus, is a pale-staining sympathetic trunk (*sy.*); just above the carotid body this rejoins the internal carotid nerve (*i.c.n.*), and in this region there is an interchange of fibres with the intraglandular plexuses of the carotid body (see next two figures). A small artery is shown, having just arisen from the ascending pharyngeal; this gives off a second branch to the upper part of the carotid body and then breaks up in the cellular perivascular tissue hereabouts. At the lower pole of the carotid body, the dark (medullated) axis cylinders of vagal fibres from the superior laryngeal nerve are seen reaching the organ; the main artery (not shown) reaches the body in this region. Bodian. × 100.

Fig. 23. H. 10/L11. In the region of the upper right quadrant of the previous figure, where sympathetic fibres from the periglandular plexus are rejoining the main internal carotid plexus. Lobules of the upper pole of the carotid body are evident and there is an interchange here, between the sympathetic trunks and the intraglandular plexuses. Bodian. × 204.

Fig. 24. H. 10/L11. The same interchange of sympathetic fibres in an adjacent section. Bodian. × 100.

Fig. 25. H. 10/L11. A part of the carotid body from fig. 22, near its upper pole. In the upper part of the figure, an obvious ganglion cell appears in the deeper part of the periglandular plexus; this cell is easily seen in fig. 22 (where it is marked with an arrow); the lobules can thus be identified. The larger lobule shows very clearly the peripheral disposition of the specific ('chief') cells around the central vascular connective-tissue core. Interlobular and perilobular nerve fibres also shown. Bodian. × 380.

Fig. 26. H. 10/L11. The same lobule at higher magnification. Note the contrast between the spherical nuclei of the principal cells, and the smaller more elongated and irregular nuclei of the 'core'. Several sinusoids are cut in transverse section; this is usual because of their highly convoluted nature. The absence of 'dark' cells is noticeable; this is so everywhere in the organ. Occasional axis cylinders can be seen among the specific cells. Bodian. × 570.

Plate 6

Fig. 27. H. 2/L11. Carmine-gelatine injection of the carotid body. A well-injected lobule is seen among empty or poorly injected ones. In the former, the central position of the sinusoids, and the peripheral, almost epithelial-like, arrangement of the 'chief' cells is obvious. In this photograph, the sinusoids appear to be cavernous spaces; although dilated, they are not as large nor as irregular as it seems—the appearance is due to their convolutions and superposition, which the photograph does not show. Haematoxylin. × 308.

Fig. 28. H. 12/L4. Indian ink injection of the carotid body. The ascending pharyngeal artery appears at the bottom left-hand corner. The carotid body is patchily injected. A well-injected group of lobules, fed by a lobular arteriole, is evident. This and the previous injection should be compared with figs. 6 and 7, Pl. 1, which show how completely the hibernating gland was injected in the same two animals. Haematoxylin. × 308.

Fig. 29. H. 10/L13. Microganglion at the lower pole of the carotid body. This section is close to fig. 23, Pl. 5, but by this time sympathetic fibres have joined the vagal fibres at the lower pole of the organ. Above, lobules of the carotid body just appear in the figure. Bodian. × 380.

Fig. 30. H. 10/L13. Superior cervical ganglion from the same section as fig. 29 to show the similarity in the appearance of the cells. Bodian. × 380.

Fig. 31. H. 4/7. Carotid body. A portion of a lobule showing a ganglion cell in intimate relation with a number of 'chief' cells. Carmine-gelatine injection. Haematoxylin. × 850.

Fig. 32. H. 4/7. Carotid body. A typical cluster of specific ('chief') cells showing their distinct outlines, spherical vesicular nuclei, and the absence of 'dark' cells. Injected (carmine-gelatine) vessels appear jet-black. Haematoxylin. × 850.

Fig. 33. H. 10/L 11. Part of a lobule of the carotid body. Above and to the left are typical 'chief' cells at the periphery of the lobule. The rest of the field shows the vascularized core. Several sinusoids (*s.*) are evident. Between them is a nucleated connective tissue in which the larger nuclei (marked by arrows) appear to correspond to those described by de Kock as 'glomus cells, type II'. Bodian. × 1250.

Fig. 34. H. 10/L 11. Part of another smaller lobule, showing at either side 'chief' cells, and between them the vascular core with a very evident sinusoid (*s.*) lined by typical endothelial cells. Just outside this is a more angular nucleus corresponding to de Kock's type II glomus cell. Bodian. × 1250.

THE CERVICAL COURSE OF THE AORTIC NERVE OF THE HORSE

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INTRODUCTION

The discovery in 1866 of the depressor or aortic nerve by Cyon & Ludwig in the rabbit was followed by numerous investigations into the cervical anatomy of this nerve in many other mammals. The main contributions were by Viti (1884), Kazem-Beck (1888), Schumacher (1902) and Perman (1924), who examined a large number of species within the Eutherian Orders, Carnivora, Perissodactyla, Artiodactyla, Rodentia, Insectivora and Primates.

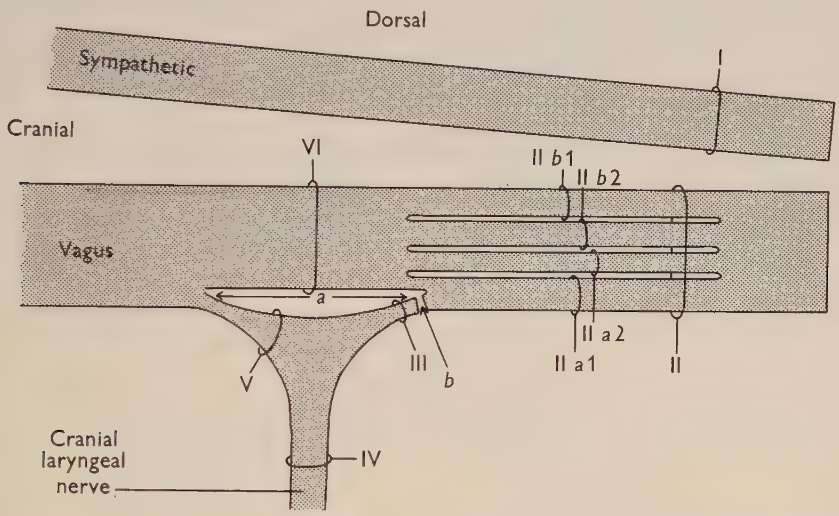
The first investigation into the equine aortic nerve was by Bernhardt (1868). He failed to find it in 'a few specimens', but remarked that the plexiform enlargement at the origin of the cranial laryngeal nerve might be homologous to the roots of the aortic nerve. In 1871 Cyon published the results of anatomical and experimental observations on a single horse. He claimed that he found a fully independent aortic nerve, with the same distinct roots of origin and separate cervical course as in the rabbit. It gave the characteristic circulatory changes on electrical stimulation. Finkelstein (1880) dissected four horses and was unable to find the nerve in two. In the others he observed it arising from two distinct and separate roots, one from the vagus and the other from the cranial laryngeal nerve, which fused to form a discrete nerve; this ran 'a short course' between the vagus and sympathetic trunk and then joined the vagus. The general articles referred to above have shown that this arrangement, which may conveniently be called the semi-independent aortic nerve, is typical of many wild and domestic Artiodactyla and Carnivora. A semi-independent aortic nerve was also found by Viti (1884) in five horses, and by Cyon (1898) and Schumacher (1902) in one specimen each. According to Schumacher the nerve remained separate inside the common vago-sympathetic connective tissue sheath for half the length of the neck; its fibres then blended mainly with the vagus, and partly with the sympathetic trunk. All these investigators relied solely on direct anatomical observations to identify the aortic nerve, except Cyon who (in both of his specimens) used electrical stimulation to confirm its identity.

There is therefore considerable agreement that the aortic nerve of the horse generally takes the semi-independent form. This conclusion was accepted by the standard text-books (e.g. Martin, 1915; Grau, 1943; Sisson & Grossman, 1953), by Heymans, Bouckaert & Regniers (1933) in their monograph, and by Barone & Valentin (1955) in a review of the cardiac nerves of the horse. Tagand & Barone (1949), however, were more cautious and simply stated that 'ces filets sensitifs sont mélangés aux autre fibres du nerf vague chez les Solipèdes'. It is evident from his monograph of 1905 that Cyon himself remained convinced that the fully independent aortic nerve was the principal form.

In preliminary dissections on a number of horses I was unable to find either a semi- or a fully independent aortic nerve. I therefore decided to reinvestigate the cervical course of the nerve in this species, using electrical stimulation to confirm its identity. Attention was concentrated on the region of the cranial laryngeal branch of the vagus because the aortic nerve fibres seemed more likely to be distinct and separate there than at any other level of the neck.

MATERIALS AND METHODS

Fifteen ponies were investigated. Under intravenous chloral hydrate anaesthesia between 5 and 10 cm. of the vagus were exposed in the region of the cranial laryngeal nerve.



Text-fig. 1. Lateral view diagram of left vagus and laryngeal plexus to show the sites of electrical stimulation. The order in which the stimulations were made is indicated by the order of the numerals.

The origin of the cranial laryngeal nerve, and the structure of the vagus and cervical sympathetic trunk for about 3 cm. on either side of the cranial laryngeal nerve, were examined. The vagus and sympathetic trunks were then cut several centimetres caudal to the cranial larygeal nerve, and stimulations from an induction coil were applied to their cranial stumps at the sites shown by the numerals in Text-fig. 1. The approximate order in which these sites were stimulated is indicated by the order of the numerals. Thus after stimulation of the sympathetic trunk at site I, the whole vagus was stimulated at site II. The base of the plexus (which occurs at the origin of the cranial laryngeal nerve) was then split from the vagus along *a* (Text-fig. 1), a procedure which usually entailed little damage to nerve fibres because of the open texture of this part of the plexus (see Pl. 1). After cutting its connexion with the vagus at B the caudal ramus of the plexus was stimulated at site III. The main trunk of the cranial laryngeal nerve peripheral to the plexus was stimulated at site IV and the cranial ramus of the plexus at site V. Next the vagus

was stimulated at the level of the mid-point of the base of the plexus at site VI. Finally the vagus was split into quarters, which were stimulated individually (sites II*a*1, II*a*2, II*b*1, II*b*2). In addition, any unidentified nerves which were found running parallel with the vagus and which might conceivably have been a fully independent aortic nerve were cut and stimulated centrally. Arterial pressure and respiration were recorded throughout on a kymograph, using a mercury manometer and a stethograph with a tambour. A reduction of arterial pressure on stimulation was the main criterion for the presence of aortic nerve fibres; additional criteria were a reduction of the pulse rate and an increase in ventilation.

The success of this method of tracing the aortic nerve fibres depends on preventing the electrical stimulus from spreading from the nerve being stimulated to other nerves, and on preserving the vitality of the nerves under stimulation. Careful precautions, one of the most essential being the use of the weakest effective stimulus, were taken to avoid these faults; in a few experiments they were not entirely successful and therefore some of the results had to be rejected.

RESULTS

The fifteen experiments are divided according to their results into three groups, A, B and C. The three experiments in group A (A1 to A3) gave full anatomical observations, but no experimental observations because the animals failed to survive the surgical preparation. The three in group B (B1 to B3) gave full anatomical observations, and also gave satisfactory results of stimulating the sympathetic trunk, the vagus immediately caudal to the cranial laryngeal nerve, and the few unidentified nerves which might have been a fully independent aortic nerve; the other experimental results were rejected because the stimulus had spread or the vitality of the nerves was impaired. The nine experiments in group C (C1 to C9) gave full anatomical and full experimental results. All the observations were made on the left side, except in Expts. A1 and A2, and C1 and C2, where they were made on the right side of the body.

Group A. In all three specimens there was a fan-like plexus, which varied only in the details of its conformation, at the origin of the cranial laryngeal nerve. That shown in Pl. 1, from specimen A3, is typical. The roots of origin of a fully or a semi-independent aortic nerve could not be seen; nor could the trunk of a semi-independent aortic nerve be found between the vagus and sympathetic inside their common connective tissue sheath.

Group B. In all three specimens the plexus was present as above, and there were no signs of the roots of either a fully or a semi-independent aortic nerve.

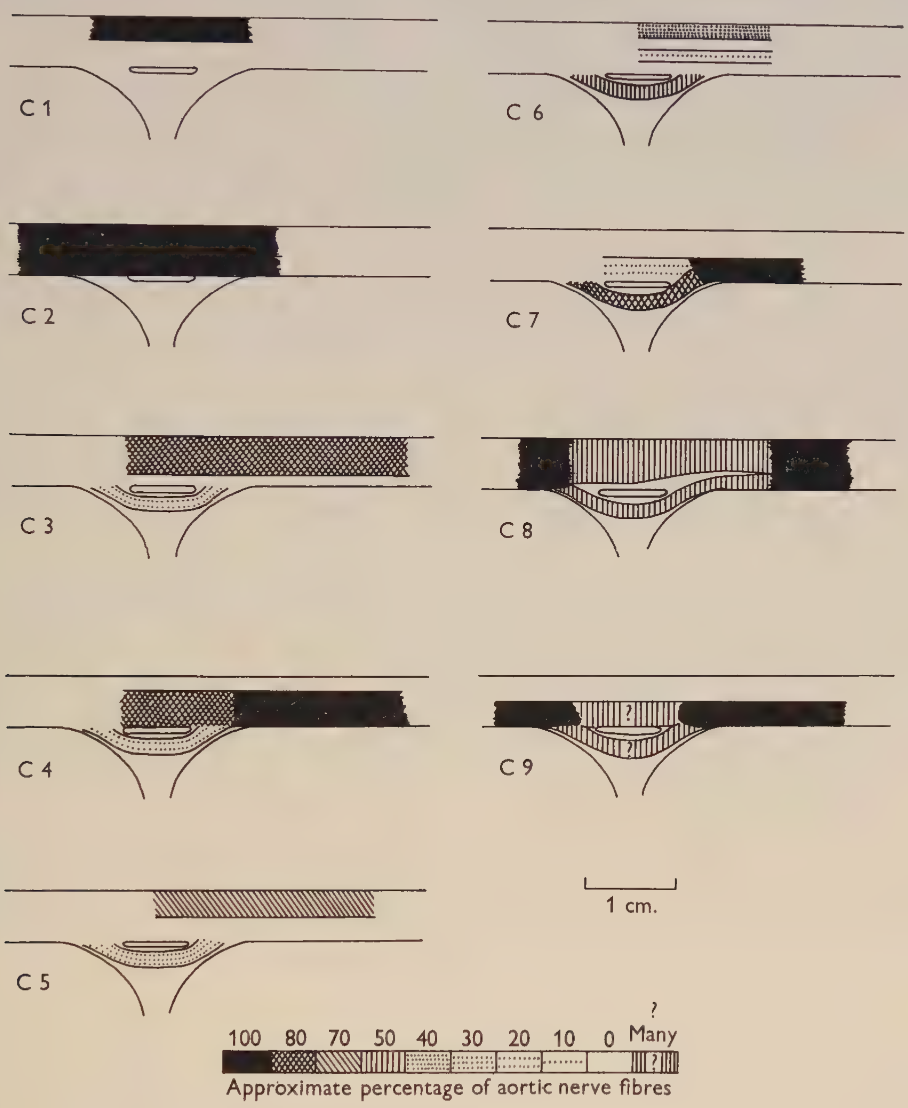
The experimental observations showed that there were no aortic nerve fibres in either the cervical sympathetic trunk or the unidentified nerves lying parallel with the vagus. They also showed that aortic nerve fibres were present in the vagus immediately caudal to the cranial laryngeal nerve.

Group C. In all nine specimens the plexus was present as above and the roots of a fully or a semi-independent aortic nerve could not be found.

The experimental observations on the sympathetic trunk and unidentified nerves showed that neither contained aortic nerve fibres.

Stimulation of the vagus at site II immediately caudal to the cranial laryngeal

nerve showed that in all nine specimens it contained aortic nerve fibres. They were scattered more or less irregularly throughout the vagus. Their positions within the vagus and their subsequent course in relation to the laryngeal plexus varied as follows.



Text-fig. 2. Lateral view diagrams of the vagus and root of the cranial laryngeal nerve to show the course of the aortic nerve fibres in specimens C1 to C9. C1 and C2 are the right vagus and the rest are the left. The approximate proportions of the aortic nerve fibres in the various parts of the nerve (estimated from the strength of the depressor effect on circulation) are shown by the shading, the key to which is on the bottom of the diagram.

In the first two experiments (C1 and C2) a marked reduction of arterial pressure and pulse rate occurred on stimulating the vagus both caudal to and level with the plexus (sites II and VI). On the other hand, no reduction of pressure occurred on

stimulation of the rami of the plexus. Therefore in these two specimens the aortic nerve fibres remained in the vagus and none were diverted across the laryngeal plexus. Stimulation of the four fractions of the vagus (II*a*1, II*a*2, II*b*1, II*b*2) induced reductions of pressure and pulse rate which indicated that the aortic nerve fibres were widely scattered throughout the vagus in Expt. C2 and confined to its dorsal half in C1 (see Text-fig. 2).

In the remaining seven specimens some aortic nerve fibres remained in the vagus and some were diverted across the laryngeal plexus (see Text-fig. 2). In two (C6 and C8) the number of fibres taking the two routes was more or less similar. In three (C3, C4 and C5) the great majority remained in the vagus: in another (C7) the great majority traversed the plexus. In the remaining experiment (C9) the proportions taking the two routes could not be estimated, but many fibres followed both routes. The fibres in the vagus were rather widely scattered in at least half and sometimes nearly all parts of the vagus. As an example of the evidence for these conclusions the essential observations made in one of the experiments (C6) are given in Table 1 and analysed as follows.

Comparison of the observations in groups 1, 2, 3 and 4, of the table show that similar reductions of arterial pressure and pulse rate were obtained from the vagus (site II), from the vagus minus those of its fibres which traversed the plexus (site VI), from the caudal ramus (site III), and from the cranial ramus (site V) (see Text-figs. 3 and 4).

This is direct evidence: first, that many aortic nerve fibres were diverted from the vagus into the caudal ramus and passed back to the vagus via the cranial ramus; and secondly, that an approximately equal number remained always in the vagus. Comparison of the respiratory changes for these four groups of observations shows that arrest in the expiratory position was the usual response to stimulation of the vagus while various changes in rate and amplitude occurred on stimulation of the rami of the plexus (see Text-fig. 4); this shows that the stimulus was not spreading between the vagus and the plexus.

The observations in group 5 show that no aortic nerve fibres passed into the cranial laryngeal nerve peripheral to its plexus. Those in group 6 show that most of those aortic nerve fibres which always remained in the vagus were confined to its most dorsal quarter, but some were in its ventro-central quarter.

DISCUSSION

There was no evidence in any of the fifteen specimens for either a fully or a semi-independent aortic nerve. Also there was no evidence for the presence of aortic nerve fibres in the cervical sympathetic trunk. On the other hand, in all of the twelve specimens in which electrical stimulation was applied aortic nerve fibres were detected in the vagus, at the level immediately caudal to its cranial laryngeal branch. Therefore it is reasonable to conclude that (at this level of the neck) either all, or at least the very great majority, of the aortic nerve fibres were contained in the vagus. Stimulation showed that, far from being condensed into one bundle, they were scattered always over at least half of the vagus and sometimes over all parts of it. The course of these fibres in the vagus, as they passed centrally from

Table 1. *The essential observations in Expt. C6*

Group of observations	Trace no.	Site of stimulation	Stimulus: Coil distance (cm.)	Stimulus: Duration (sec.)	Effect on circulation: Change in arterial pressure (%)	Effect on circulation: change in pulse rate (%)	Effect on respiration
p 1. On the le vagus	10	II	10	25	−24	−11	Arrest in expiratory position
	12	II	9	25	−19	−12	Great reduction in amplit. and rate
	24	II	10	20	−35	− −*	Arrest in expiratory position
	25	II	10	20	− 9	−*	Arrest in expiratory position
					av. = −22		
p 2. On the us minus fibres ersing the us	19	VI	10	20	−19	−17	Arrest in expiratory position
	20	VI	9	20	−19	−20	Arrest in expiratory position
					av. = −19	av. = −18	
p 3. On the dal ramus of the us	13	III	10	20	−21	−34	Small amplit. reduct.; small rate incr.
	14	III	12	27	−20	−28	Small amplit. reduct.; small rate incr.
	23	III	10	20	−20	−37	Great amplit. reduct.; rate unchanged
					av. = −20	av. = −33	
p 4. On the ial ramus of the us	18	V	10	15	−34	−45	Great amplit. reduct.; small rate reduct.
	21	V	10	30	− 7	−17	Great amplit. reduct.; rate unchanged
	22	V	10	30	−20	−29	Great amplit. reduct.; rate unchanged
					av. = −20	av. = −30	
p 5. On the ngeal nerve pheral to plexus	15	IV	10	30	− 3	−12	Great amplit. reduct.; small rate reduct.
	16	IV	10	20	+ −0	+ −0	Small amplit. reduct.; rate unchanged
	17	IV	10	35	+ −0	+ −0	Small amplit. reduct.; small rate incr.
p 6. On the tral (II*a*1), tro-central 2), dorso- tral (II*b*2) and sal (II*b*1) rters of the us	29	II*a*1	11	25	+ −0	+ −0	Small amplit. reduct.; rate unchanged
	33	II*a*1	10	25	+ −0	+ −0	Small amplit. reduct.; rate unchanged
	30	II*a*2	11	25	− 7	−11	Amplitude and rate unchanged
	34	II*a*2	10	25	− 8	−11	Small amplit. reduct.; rate unchanged
	31	II*b*2	11	20	+ −0	+ 5	Amplitude and rate unchanged
	35	II*b*2	10	35	− 4	− 6	Amplitude and rate unchanged
	32	II*b*1	11	25	−22	−28	Arrest in expiratory position
	36	II*b*1	10	20	−28	−28	Arrest in expiratory position

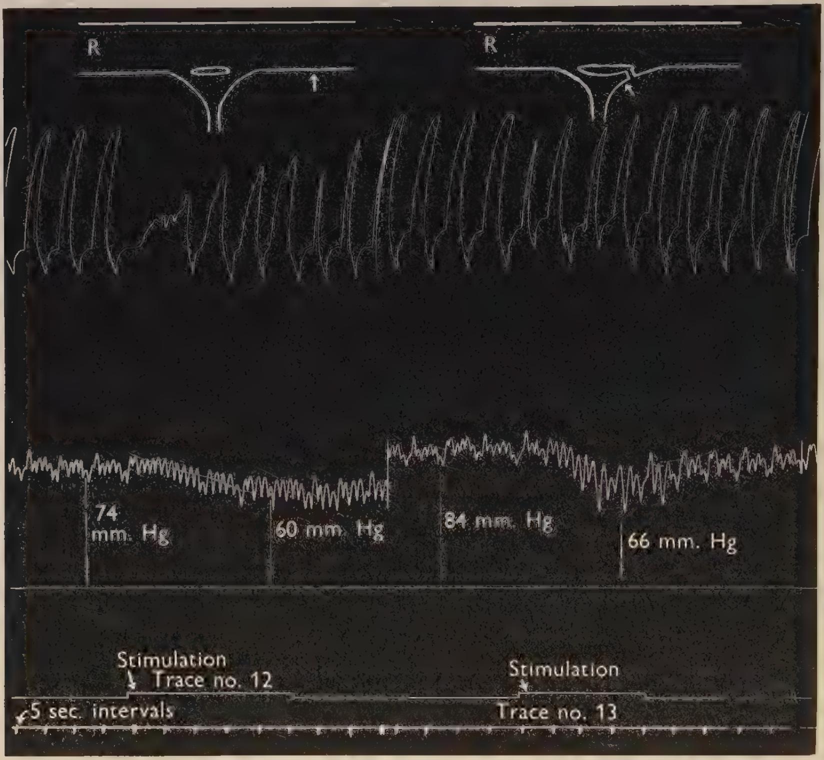
The entries under 'Trace no.' show the order in which the observations were recorded. Trace nos. 1–9, and 11, 26, 27 and 28 are omitted; these were stimulations of the sympathetic trunk and unidentified nerves (which gave no circulatory effect), and test stimulations of the vagus at coil distances over 11 cm. Amplit. = amplitude; incr. = increase; reduct. = reduction; av. = mean.

* In places the pulse rate could not be counted, but a moderate (−) or great (− −) reduction was evident.

this level to above the laryngeal nerve, varied in the nine specimens which were fully investigated. Either they remained entirely in the vagus (in two specimens), or they divided into two streams, one remaining in the vagus and the other being temporarily diverted from the vagus to traverse the plexus at the origin of the cranial laryngeal nerve (seven specimens).

The two specimens in which they were entirely confined to the vagus were examined on the right side, whereas the other seven were all investigated on the left. This suggests the possibility that the course of the aortic nerve fibres may differ on the two sides of the body. It has been found in a number of mammals that the left aortic nerve tends to be more independent from the vago-sympathetic trunk than the right; for example, Bernhardt (1868), Kazem-Beck (1888), and Schumacher

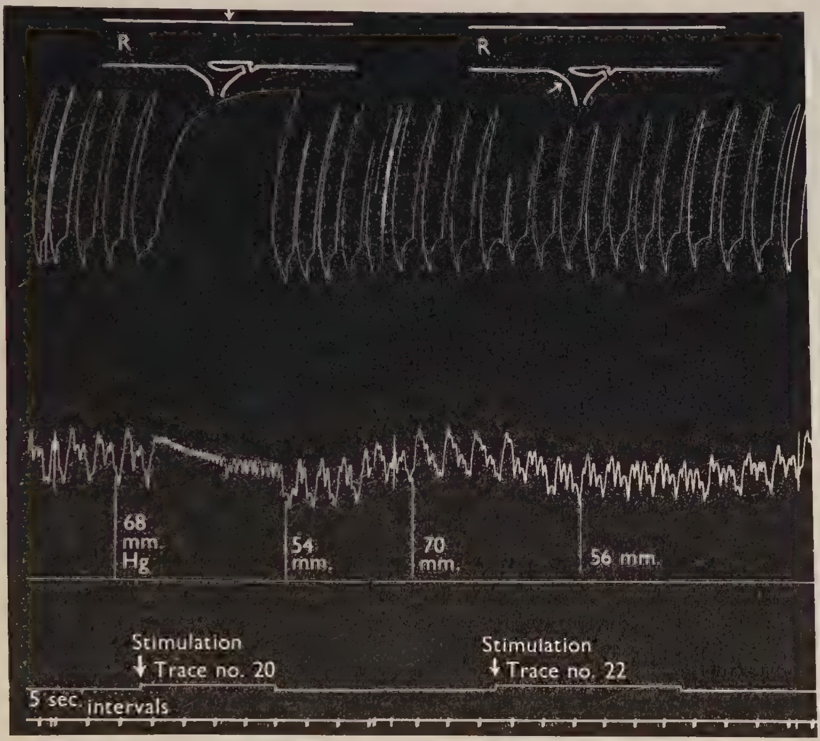
(1902) and others, have shown that in the cat the left aortic nerve is fully independent much more often than the right. In the horses investigated here the restriction of the aortic nerve fibres of the right side to the main trunk of the vagus, and the diversion of a proportion of those of the left side across the base of the cranial laryngeal nerve, may be an expression of the same tendency for a relatively greater independence on the left side.



Text-fig. 3. Trace nos. 12 and 13 from Expt. C6, recording respiration (above) and arterial pressure (below). The site of stimulation is marked by an arrow on the two diagrams of the vagus; their orientation is as in Text-fig. 1, R meaning rostral. Trace no. 12 is stimulation of the whole vagus at site II; trace no. 13 is stimulation of the caudal ramus at site III. The arterial pressures are written in. The changes in pulse rate are as follows: Trace no. 12: rate before stimulation, 66 per minute; rate after stimulation, 58. Trace no. 13: rate before stimulation, 68 per minute; rate after stimulation, 45.

As stated above, all those who claimed to have found the nerve (i.e. Cyon 1871, 1898; Finkelstein, 1880; Viti, 1884; Schumacher, 1902) described it as a discrete nerve which arose centrally by a distinct root from the vagus, or cranial laryngeal nerve, or both, and could be isolated for a variable length inside (or even outside) the vago-sympathetic sheath. In the horse the vagus consists of rather loosely knit fascicles, many of which can quite easily be dissected away from the rest of the trunk, and the laryngeal plexus can also easily be broken down into its component fascicles. In searching for a semi-independent aortic nerve, the form which had been shown to occur commonly in mammals, the earlier workers may have created

artefacts by trying to remove the connective tissue sheath from the vagus and plexus; these would have been rather deceptive in the absence of any functional test of their identity, such as electrical stimulation. Cyon alone used such a test. Yet he convinced himself that both of the horses which he investigated possessed a fully independent aortic nerve. The fact that no other worker has been able to repeat this finding strongly suggests that he was mistaken, for, as Bernhardt (1868)



Text-fig. 4. Trace nos. 20 and 22 from Expt. C6, as in Text-fig. 3. Trace no. 20 is stimulation of the vagus at site VI; trace no. 22 is stimulation of the cranial ramus at site V. The changes in pulse rate are as follows. Trace no. 20: rate before stimulation, 90 per minute; rate after stimulation, 72. Trace no. 22: rate before stimulation, 96 per minute, rate after stimulation, 68.

put it, 'considering the colossal dimensions of these animals...it is unlikely that the nerve could be overlooked'. Cyon too could have been misled by an artefact, though in this instance it was most probably the spread of the stimulus to the vagus; this interpretation of Cyon's findings is supported by his failure to mention any details of his stimulation technique, or precautions to prevent spreading of the stimulus.

SUMMARY

1. Nearly all previous accounts agree that in its cervical course the aortic nerve of the horse is either fully independent as in the rabbit or semi-independent as in many other mammals.

2. In an investigation of fifteen horses there was no evidence for either of these forms. In twelve, electrical stimulation of the vagus showed that the aortic nerve

fibres were contained in the vagus, in the region about 3 cm. immediately caudal to the cranial laryngeal nerve; none were in the cervical sympathetic trunk. In nine of these, electrical stimulation revealed the course of the aortic nerve fibres as they ascended centrally to a point about 2 cm. above the cranial laryngeal nerve: in two specimens all the fibres were confined to the vagus; in the other seven they were partly contained in the vagus and partly diverted from the vagus to pass across the plexus which occurs at the origin of the cranial laryngeal nerve. The two specimens in which the fibres were entirely confined to the vagus were investigated on the right side of the body, and the other seven were investigated on the left.

3. It is suggested that there may be a difference on the two sides of the body arising from a tendency in mammals for the left aortic nerve to be more independent than the right.

4. The claim of previous workers to have found a fully or semi-independent aortic nerve is ascribed to the creation of artefacts through dissection of the vagus or spreading of electrical stimuli.

I am very grateful to Prof. E. C. Amoroso for supervising this enterprise, Dr H. Rosenberg for his advice, and Dr F. R. Bell for doing an equal share of the experimental work. I also thank A. Goffin, M. Bowlding, A. G. Hanks and A. Coombs for technical assistance.

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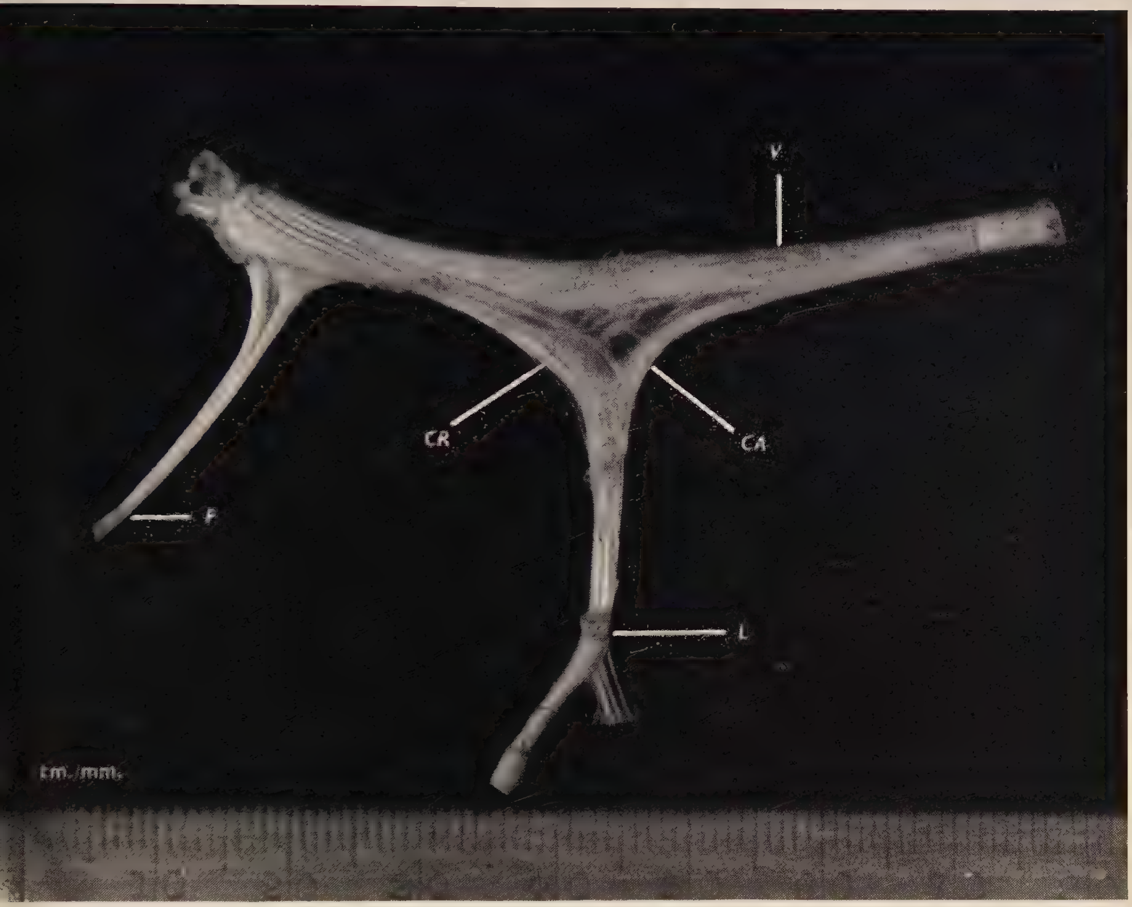
EXPLANATION OF PLATE

Lateral view of the left vagus of specimen A3. The plexus at the root of the cranial laryngeal nerve is typical. V = vagus; CA = caudal ramus, CR = cranial ramus, of plexus; L = cranial laryngeal nerve; P = pharyngeal nerve.



KING—The cervical course of the aortic nerve of the horse

(*Facing p.* 236)

THE DEVELOPMENT OF THE BLOOD SUPPLY OF THE PITUITARY IN THE ALBINO RAT, WITH SPECIAL REFERENCE TO THE PORTAL VESSELS

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Portal vessels connecting the capillary network of the median eminence with the sinusoids of the pars distalis are a constant feature in vertebrates (Green, 1951). Their anatomical arrangement has encouraged many workers to believe that they are an essential link in a control which the hypothalamus is presumed to exercise over the pars distalis, and for which there is no apparent neurological basis (Harris, 1948, 1955). This functional inference is, however, essentially based on argument by exclusion, and a critical test has now proved that in the ferret a direct vascular connexion between the base of the brain and the pars distalis is 'neither sufficient, other things being equal, nor necessary, for positive responses of the pars distalis' (Zuckerman, 1955).

The history of this chapter of research is not the first to reveal the caution which needs to govern all inferences about function from structure. What is surprising is that little recourse has been made to embryological study in order to obtain a clearer understanding of the definitive anatomical arrangement of the portal vessels.

The adenohypophysis develops from Rathke's pouch and presumably derives its original blood supply from the roof of the primitive pharynx. On the other hand, the neurohypophysis is an outgrowth from the floor of the third ventricle, and when it first appears must be supplied by the same system of vessels which also supply the hypothalamus. Such limited information as exists supports the view that at an early state of development the adenohypophysis loses its primitive blood supply from the pharynx, and new vascular channels—the portal vessels—form along the length of the pituitary stalk and connect the pars distalis with the hypothalamus.

Few authorities agree, however, about the precise nature of this process. For example, 'Espinasse (1933) concluded from a study of five human embryos ranging from 1 to 5 months in age that the portal vessels are originally part of the arterial supply to the hypothalamus, and that distally they become incorporated as sinusoids in the upward-growing adenohypophysial tissue. On the other hand, Wislocki (1937) holds that the vessels supplying the human adenohypophysis are of pial origin. According to this worker, the vessels at first form a normal systemic blood supply, but with the development of lateral venous connexions between the pars distalis and the cavernous sinuses the pial venules regress, leaving only their distal ends, which later form the portal vessels of the adult.

Assenmacher (1951), Wingstrand (1951) and Grignon (1954) all agree that in birds the developing neuro- and adenohypophysis are surrounded by a single capillary plexus. Vessels from this plexus, and accompanying mesenchyme, later invade the

entire hypophysis. When the pituitary stalk is formed, the capillary plexuses in the pars distalis and the base of the median eminence separate but remain connected by stretched capillaries. These develop into the portal vessels.

In view of the paucity of information about the formation of the hypophysial vessels, it was decided to carry out further investigations, using the albino rat. The rat is well suited for this purpose, first, because the nature of the portal vessels can be well demonstrated within its long pituitary stalk, and second, because it has often been used in studies of pituitary function.

MATERIAL AND METHOD

Material

All the animals used in this study were bred from virgin albino rats belonging to a strain established in this laboratory. Insemination was taken as having occurred at the midnight prior to the finding of sperm in vaginal smears, which were taken each morning. Birth of the offspring occurred almost invariably 23 days later, and this was defined as 'day one' *post natum* in the life of the young rat.

The animals were maintained under standard laboratory conditions, with food and water supplied *ad lib.* Each litter was caged separately.

The foetal rats studied ranged from 14 to 22 days after insemination, while the ages of the post-natal rats were 1, 2, 3, 5, 7, 10, 18, 21, 40 and 50 days. At least six specimens were studied for each age-group.

Methods

Using Harris's perfusion method (1950), all rats were injected through the left ventricle with a solution composed of equal parts of mandarin black waterproof carbon ink (Winsor and Newton), and 5 % sodium citrate, in sufficient quantity to fill the vascular system. Only preparations which appeared to be completely perfused were retained.

After injection, all specimens were fixed in 10 % formol saline. Embryos were fixed *in toto*, but in post-natal rats the mandible and calvarium were removed before fixation of the head. The specimens were decalcified in a buffered solution of formic acid and sodium citrate, and then washed for 24 hr. in running water. They were trimmed to leave a single block which comprised the base of the brain, including the hypothalamus and pituitary, together with adjacent skull.

Specimens representing each age studied were embedded in both paraffin and celloidin and cut coronally and sagittally. Paraffin sections were cut at $8\,\mu$ and stained with Weigert's haematoxylin and chromotrope, or by Cleveland & Wolfe's technique (1932), or by Masson's trichrome stain. The celloidin sections—$50\,\mu$ or $100\,\mu$ in thickness—were cleared in a solution of beechwood creosote and carbol xylol and then mounted.

RESULTS

Fourteen days after insemination

The buccal component of the pituitary forms an open, vertically disposed pouch in intimate contact with the base of the diencephalon. The neurohypophysis is clearly defined but smaller. The first signs of the tuberal processes can be seen as prolifera-

tions of the antero-inferior aspect of Rathke's pouch. The whole pituitary complex is surrounded by a plexus of blood capillaries lying in the mesenchyme around Rathke's pouch. The neurohypophysis contains a few capillaries.

Fifteen days after insemination

Rathke's pouch is now closed and its lumen restricted to the upper half. The pouch is still connected to the roof of the mouth by a short vertical stalk of cells lying in connective tissue containing many capillaries. The tuberal process has increased considerably in size, and its forward growth forms a shelf of epithelial tissue, resulting in the formation of a superior recess (Atwell, 1918) containing capillaries and connective tissue (Pl. 1, fig. 1).

The adenohypophysis is entirely surrounded by connective tissue. A very thin layer of pial mesenchyme separates the adenohypophysis from the neurohypophysis and the median eminence. The connective tissue surrounding the rest of Rathke's pouch is the forerunner of the adult dura.

The neural process of the neurohypophysis has also increased in length and is subdivided into cords and lobules of cells. It is vascularized by pial vessels from all sides, most of them entering the anterior and postero-superior aspects of the neurohypophysis.

Sixteen days after insemination

Rathke's pouch has undergone a rotation on its horizontal axis through approximately 90 degrees. The stalk is limited to a narrow strand of cells extending from the roof of the mouth to the under-surface of the tuberal process, which appears to receive blood from the plexus of capillaries within the stroma of the stalk.

The relation of the adenohypophysis to its surrounding connective tissue is of special interest and importance. The anterior aspect of the growing adenohypophysis is pierced by pial tissue, leading to extensive infiltration of the gland. A core of connective tissue is found in the anterior part of the tuberal process, which eventually links up with similar tissue entering the tuberal process from below. Except in the extreme anterior parts of the tuberal process, blood vessels are present everywhere in this connective tissue.

Injected specimens show no ink in the vessels actually within the adenohypophysis, although it is clearly present in the surrounding plexuses.

Seventeen days after insemination

The stalk of Rathke's pouch remains only in the sphenoid anlage as a thin strand of cells surrounded by dense connective tissue containing a few capillaries (Pl. 1, fig. 1).

As the tuberal process continues to grow forwards on the under-surface of the brain, it splits the mesenchyme into two unequal layers, the thicker one passing below to form a condensation on the upper surface of the sphenoid procartilage, and the thinner one passing down to form a diffuse layer in continuity with the tissue of 'Atwell's recess' and that between the adenohypophysis and pars nervosa. The upper layer contains many capillaries which derive blood from branches of the internal carotid arteries. This is the earliest stage at which it is possible to reach a view about the direction of the flow of blood in and around the developing pituitary gland.

The anterior part of the tuberal process is now well formed and more solid in consistency. Its cranial edge lies close to the base of the brain, being separated from it by the thin layer of mesenchyme and capillaries already described. The tuberal process is being invaded by mesenchyme, and some specimens contain a few capillaries.

Eighteen days after insemination

The stalk of Rathke's pouch has disappeared (Pl. 1, fig. 2).

The tuberal process continues its forward growth and the cranial part (pars tuberalis) lies close to the developing tuber cinereum but separated from it by the thin layer of vascularized pia mater, which now extends over an appreciable area. Injected specimens show that all blood enters the adenohypophysis through the vessels in the layer of pia mater between the pars tuberalis and the tuber cinereum, and through vessels in the connective tissue of Atwell's recess. The only exception is that a small amount of blood enters the inferior aspect of the adenohypophysis by way of vessels passing through the gap in the sphenoid which remains in the pathway of Rathke's stalk.

Nineteen days after insemination

The sphenoid forms a continuous plate of cartilage and bone below the pituitary. Although the few degenerating blood vessels previously associated with the stalk can still be seen, the foramen which marked the route taken by the stalk of Rathke's pouch is now closed by ossifying tissue (Pl. 1, fig. 3).

The layer of pia covering the upper surface of the pars tuberalis contains a plexus of capillaries denser than in any other region of the adjacent pia. This supratuberal plexus of vessels does not penetrate the tuber cinereum above, though it is connected to vessels in the pars tuberalis below.

Twenty days after insemination

No blood vessels now connect the pituitary gland with the roof of the mouth.

Careful study of the supratuberal plexus of capillaries reveals that it is composed of vessels running mainly cranio-caudally to drain into the pars distalis. Some of these capillaries are remarkably long and straight. Blood from this plexus also passes to the neural lobe.

The neurohypophysis has become considerably elongated, and it is possible to distinguish neural lobe and neural stalk. The latter is becoming increasingly encircled by lateral extensions from the pars tuberalis.

Twenty-one and twenty-two days after insemination

The most important change from the previous day is in the condition of the supratuberal plexus. Hypophysial portal vessels appear for the first time (Pl. 1, fig. 4; Pl. 2, fig. 5). They are represented in the caudal region of the plexus by one to three vessels of larger diameter than any others. They run caudally either within the pars tuberalis or through the connective tissue separating the *partes tuberalis et neuralis* to empty into the sinusoidal vessels within the adenohypophysis which now receives all its blood via the supratuberal plexus of vessels.

The neural process is no longer separated from the pars intermedia by connective tissue. The pia mater surrounding the process has also disappeared and dura lies in direct contact with the neural tissue. Though obtaining some blood from the supratuberal plexus, the neural process receives its major supply from a vessel or vessels entering the dura at its postero-superior pole.

Post-natal development

The rich capillary network seen in the median eminence of the adult does not begin to develop until the 5th day after birth, when a number of small capillary loops invaginate the median eminence from the supratuberal plexus (Pl. 2, figs, 6, 7). This process continues by a series of increases in number, complexity and size of the invaginating loops until 40 days after birth, when the adult condition is established (Pl. 2, fig. 8).

DISCUSSION

The present findings agree in general with those of other studies on the vascular development of the hypophysis in birds and man. The portal vessels of the adult owe their presence and disposition to specific morphological changes which take place in the hypophysial area during the course of its development.

In its earlier stages, the whole pituitary complex is surrounded by a plexus of meningeal vessels. The plexus is connected to vessels lying in the roof of the primitive pharyngeal cavity. With subsequent development of the sphenoid this connexion is lost.

Growth of the tuberal process of the adenohypophysis leads to the trapping of part of the peri-hypophysial capillary plexus beneath the median eminence (the supratuberal plexus). Subsequently, when the major part of the pituitary and its accompanying blood vessels become separated from the median eminence and supratuberal plexus by the development of the pituitary stalk, the vessels in the intervening area become stretched and form the portal vessels connecting the two consolidated capillary plexuses of the pars tuberalis and pars distalis.

These 'portal vessels' connect the primary capillary plexus in the median eminence and pars tuberalis not only with the sinusoids of the pars distalis but also with the capillaries of the neural lobe. This is not surprising in view of the way the pituitary blood supply develops from the perihypophysial capillary plexus. The fact that there is this interconnexion has been previously reported by Landsmeer (1947, 1951), Barrnett & Greep (1951), and Daniel & Pritchard (1956) as a result of studies on rats. In man the pituitary gland is supplied from below by two inferior hypophysial branches of the internal carotid arteries which immediately enter the neural lobe, and from above by a series of superior hypophysial vessels which spring from the circle of Willis and which pass to the pars distalis either directly or via the primary capillary network in the median eminence. According to McConnell's (1953) recent description, branches of both the superior and inferior hypophysial arteries occur in the lower part of the pituitary stalk. These branches, which are referred to as 'parallel vessels', are said to supply blood to the sinusoids of the pars distalis as well as to the capillaries of the neural process. In their still more recent account of the vasculature of the human pituitary, Xuereb, Pritchard & Daniel (1954*a*, *b*) also

describe special anastomotic channels between the superior and inferior hypophysial arterial systems.

In man, portal vessels first appear about the 4th month of intrauterine life, and the tufts and loops of the adult primary capillary plexus do not develop until later, during the second half of foetal life (Niemineva, 1950*a*, *b*). The relative length of the interval between the appearance of the portal vessels on the one hand, and the primary capillary plexus on the other, is even more marked in the rat, in which portal vessels are first seen 3 days before birth, and in which there is no sign of the primary capillary plexus until a week later (i.e. 5 days after birth, when small capillary loops arising from the supratuberal plexus are seen to penetrate into the base of the median eminence). The fully developed condition is not achieved until some 40 days later.

There is evidence that the pituitary becomes physiologically active before the portal vessels develop (Wilson, 1952; Jost, 1953*a*, 1954). Thus, Jost & Gonse (1953) have shown that the anterior pituitary is very rich in periodic–acid–Schiff positive material (which they state might well be F.S.H. and T.S.H.) during the 22nd and 23rd days of gestation in the rabbit. Hypophysectomy by decapitation on either of these 2 days leads to the retarded development of the thyroid and testes. A similar retardation is also noted in rabbits that have been operated upon prior to these days, when they reach the 22nd or 23rd day of gestation (Jost, 1953*b*). Rabbits operated upon after this date do not show the same changes.

Decapitation of the foetal rat before sexual differentiation leads in 3 days to a reduction in the size of the accessory reproductive organs. Decapitation after differentiation has occurred leads to a reduced number of interstitial cells in the testes (Jost & Cologne, 1949). Decapitation also leads to adrenocortical atrophy, which can be prevented by injecting the rat foetus with ACTH (Kitchell & Wells, 1952). Correspondingly, X-ray destruction of the pituitary in the mouse foetus results in a decreased number of germinal cells in the gonads, a small thyroid with follicles lacking colloid, and atrophic adrenals (Raynaud, 1950; Raynaud & Frilley, 1950). All this would seem to indicate, first, that the pituitary functions before birth, and second that it does so in the absence of the distinctive vascular arrangement of the adult median eminence.

SUMMARY

1. The development of the blood supply to the pituitary has been studied, special attention being paid to the hypophysio-portal vessels.
2. Albino rats were studied over an age range of 60 days; from 14 days after insemination to 50 days after birth.
3. Investigation has shown that the portal vessels may first be seen 3 days before birth, whilst the first signs of the primary capillary plexus are not evident until 5 days after birth.
4. Portal vessels were found passing to the neural lobe.
5. The findings are discussed, and it is concluded that in the albino rat, the foetal pituitary can function in the absence of the distinctive vascular arrangement of the adult median eminence.

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EXPLANATION OF PLATES

Photomicrographs of the pituitary in the foetal and adult rat. All sections in the median sagittal plane. Stained sections ('H. & Ch.'): 8μ, haematoxylin and chromotrope. Injected sections ('celloidin'): 100μ, injected, cleared celloidin.

PLATE 1

Fig. 1. Sixteen days after fertilization. Pituitary now rotated into horizontal plane. Tuberal process growing forward leading to formation of Atwell's recess and subsequent invasion of adenohypophysis by connective tissue. (H. & Ch., ×75.)

Fig. 2. Eighteen days after fertilization. The supratuberal plexus is now developing. Blood vessels in the pars distalis are of significantly greater diameter than in any other areas and begin to assume appearance of sinusoids. Pars intermedia relatively poorly vascularized compared with other parts. (Celloidin, ×90.)

Fig. 3. Nineteen days after fertilization. Pituitary assuming adult configuration, well-developed plate of pars tuberalis beneath median eminence. (H. & Ch., ×63.)

Fig. 4. Twenty-two days after fertilization. First appearance of portal vessels connecting the supratuberal plexus to sinusoids of pars distalis. (H. & Ch., ×47.)

PLATE 2

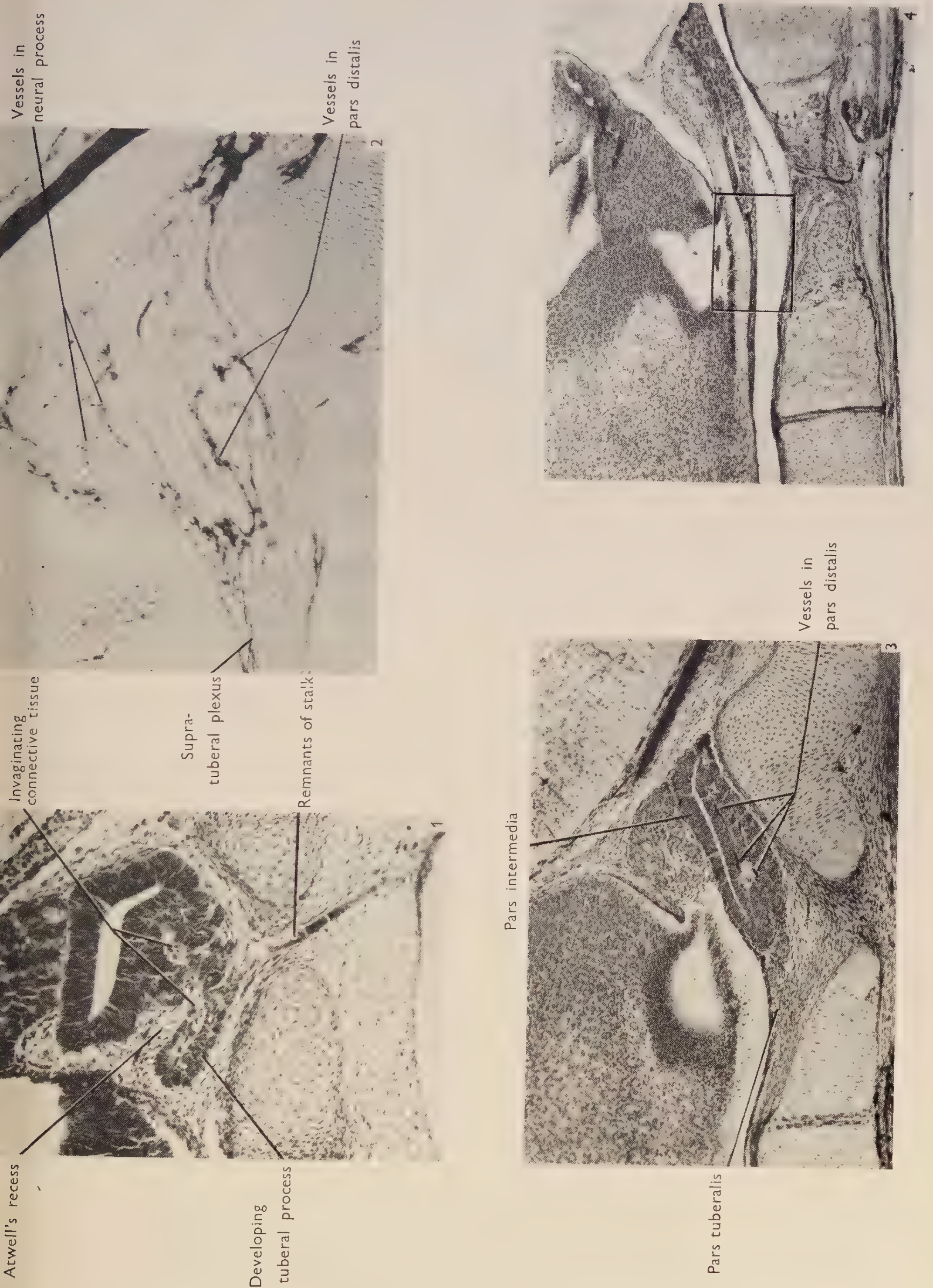
Fig. 5. Part of fig. 6, showing portal vessel. ×280.

Fig. 6. Five days after birth. First appearance of capillary loops in median eminence. The plexus from which they arise still lies predominantly *between* neural tissue of median eminence and pars tuberalis. (Celloidin, ×50.)

Fig. 7. Part of fig. 8, showing early tuft formation in median eminence. ×330.

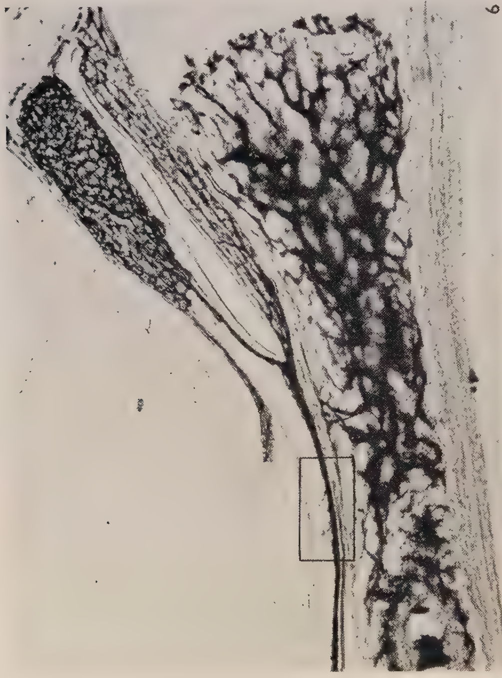
Fig. 8. Condition in adult rat. The supratuberal plexus has now greatly expanded, invading both median eminence and pars tuberalis. It is present all round the stalk (e.g. at *X*, where a particularly well-developed complex of sinusoidal vessels is seen in the neural portion of the pituitary stalk).

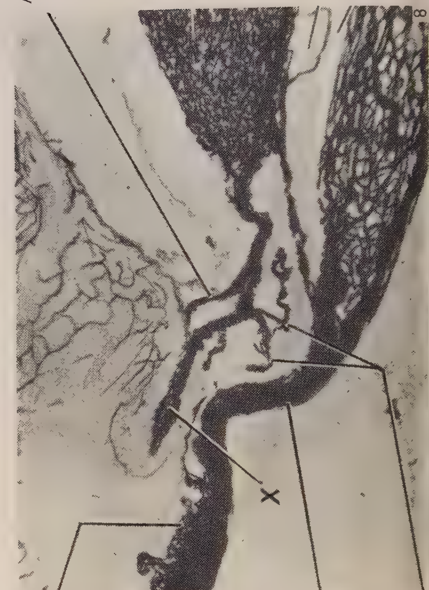
The primary capillary plexus is continued around the neural process where it lies adjacent to pars intermedia. (Celloidin, ×42.)



GLYDON—DEVELOPMENT OF HYPOPHYSIAL PORTAL VESSELS

(*Facing p.* 244)





GLYDON—Development of hypophysial portal vessels

THE BLOOD VESSELS OF THE ADULT MAMMALIAN SPLEEN

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The present study was undertaken to determine whether in the adult spleen both arterial capillaries and venous sinuses open into the pulp spaces, their walls becoming continuous with the reticulum bounding the pulp spaces, as would be expected from the embryological findings of Lewis (1956). Also, the possible existence of arterial loops and arcades, such as those found in the embryo, had to be investigated in the adult. The conflicting opinions held with regard to the nature of the adult mammalian splenic circulation have also been reviewed by Lewis (1956).

METHODS

Cat, rabbit, dog and sheep spleens, removed from freshly killed animals, and human spleens, removed at post-mortem, were used. Neoprene casts of the vascular system were prepared by injecting either via the arteries or the veins at physiological pressures, and corroding away the tissue with acid.

To check the arterial vascular pattern as seen in the neoprene casts, micropaque solution was injected through the splenic arteries in a number of spleens, and radiographs taken.

To obtain histological material for correlation with the neoprene casts the spleen was injected with 10% formalin solution via the arteries, thus washing out much of the blood through the veins and distending the organ to something approaching its size *in vivo*. Following fixation in 10% formalin and embedding in paraffin wax, sections of 20μ were cut and heavily stained with Heidenhain's iron haematoxylin. These sections provided a clear picture of the architecture of the spleen. In addition 8μ sections of some distended spleens were stained with Weigert's haematoxylin and Van Gieson stain, haematoxylin and eosin, Weigert's elastic stain, Leishman's stain and Giemsa stain.

OBSERVATIONS

The arterial pattern

As seen in the neoprene casts of all species, the arterial branches after entering the spleen form a simple arborizing pattern down to the smallest capillaries. These arterial branches present no large loops giving rise to smaller branches, nor are large anastomoses between neighbouring branches found, except very rarely. Such an anastomosis has been found only in one cat spleen, forming an arterial arcade giving rise to smaller branches. The arteries frequently present a spiral or corkscrew form or have localized constrictions along their length (Pl. 1, fig. 3). The smallest branches, as can be seen in a teased preparation of an arterial-injected specimen, terminate in small nodular masses of neoprene (Pl. 1, fig. 5). This gives the whole

cast of such a spleen a fluffy, irregular form traversed by definite arteries and their fine branches. The same simple arborizing pattern of the arterial branches is seen in the arteriograms.

Histological sections cut at $20\,\mu$ thickness and stained with Heidenhain's iron haematoxylin show clearly the form of those regions where the smallest arterial branches of the neoprene casts terminate in irregular masses. Arterial capillaries after leaving a splenic ellipsoid, or Schweigger–Seidel sheath, in those spleens possessing them, terminate in a funnel-shaped fashion in the pulp. The endothelial cells of this terminal portion diverge from one another, stomata appear and the walls assume a lace-like form which gradually merges with the reticular tissue of the pulp (pl. 1, figs 6, 7).

The venous pattern

Neoprene casts of spleens injected through splenic veins show a varying degree of elaboration of venous sinusoids. The dog spleen shows the highest development of sinusoids, the collecting veins breaking up into a complicated tangled mass of fine tributaries, the sinusoids, which, however, do present a more or less regular radiating pattern as seen from the surface of a cast (Pl. 1, figs. 2, 4). These sinusoidal casts end blindly with rounded or tapering terminations or as small nodular masses similar to those seen at arterial terminations. Neoprene casts of cat spleens, similarly prepared, show a different form—here the larger veins branch and quickly terminate as interconnected, irregular, nodular masses of neoprene and not as definite vessels as in the dog (Pl. 1, fig. 1). The sheep has a rather intermediate form, irregular masses often following large veins, though often quite small sinusoids are reached before terminating in similar aggregations. The human spleen possesses a sinusoidal development approaching that of the dog, though the sinuses appear to be more nodular and varicose and form a more irregular pattern.

Sections of spleens of $20\,\mu$ thickness stained with Heidenhain's iron haematoxylin show clearly the form of the sinusoid walls as described by Mollier (1911). In the dog the walls are composed of a lattice-like arrangement of elongated cells with longitudinally running stomata between, though cross-connexions of cytoplasm do bridge across the stomata (pl. 2, fig. 12). In the sheep the walls are more irregular, formed by a sieve-like, nucleated, syncytium with large stomata and a less apparent longitudinal orientation. The species variation in extent of development of sinusoids can be seen in such sections; for example, many more are apparent among the pulp spaces of the dog spleen than in the sheep, while none are to be seen in the cat spleen. The pulp of the sheep spleen, although possessing few venous sinusoids, is traversed by numerous smooth muscle bundles (Pl. 2, fig. 13). The sinuses of the species examined can be seen to terminate in the pulp in similar fashion to the arterial capillaries. Their cellular walls merge with the reticular tissue of the pulp so that the sinuses are continuous with, or open into, the pulp spaces (Pl. 2, figs. 8, 9).

The splenic ellipsoids

In the dog, as seen in $20\,\mu$ sections stained with Heidenhain's iron haematoxylin, these are highly developed (Robinson, 1926) and ensheath the arterial capillaries a short distance prior to their opening into the pulp. The ellipsoids in this species regularly have two or more sinusoids in close relation to them, the sinusoids being

intimately applied to their surface (Pl. 2, fig. 10). In many places portions of the typical sinusoid wall are seen as the sinuses approach or leave the surface of the ellipsoid. Where the arterial capillary or a branch of it leaves the ellipsoid to pass into the pulp, it passes between and not through the enveloping sinuses. Haematoxylin and eosin or Weigert's haematoxylin and Van Gieson sections of 8μ thickness show more clearly the structure of the ellipsoids. They consist of a fairly closely packed aggregation of cells identical with the cells of the reticular tissue of the pulp, and there are small spaces between the cells giving the ellipsoid a spongy texture (Pl. 2, fig. 11). The enveloping sinusoids are intimately applied to their surface and the arterial capillary traverses the centre of the ellipsoid. In some of the Heidenhain's haematoxylin 20μ sections where the arterial capillary leaving the ellipsoid is in close relation to one of the approaching or leaving sinuses, the appearance may be given of an arterial capillary opening into a sinus; however, the true relationship of the vessels, one of proximity only, is seen on close examination. No case of an arterial capillary really opening into a sinus was observed.

In the sheep the ellipsoids are poorly developed, consisting of small aggregations of reticular cells surrounding the arterial capillaries fairly close to their terminations. No sinuses are applied to their surfaces and they are simply surrounded by the pulp spaces, their cells being continuous with the reticular tissue of the pulp (Pl. 2, fig. 13).

In the cat the ellipsoids are well developed and are distributed in the pulp as in the sheep, with no surrounding sinusoids.

DISCUSSION

The arteries in the adult mammalian spleen show a simple arborizing pattern down to the finest arterial capillaries, having no looped or arcade form as is seen in the embryo. In only one specimen (a cat spleen) has an anastomotic arcade been seen in an adult spleen. The looped system of the embryo would seem to be a device for equalizing pressure in those arterial branches opening into the primitive pulp spaces during the phase when these are being distended with blood, thus producing uniform filling. Following this phase, as the organ grows, the branches arising from the loops must develop progressively in an arborizing manner, the loops becoming more and more insignificant and ultimately disappearing.

The spiral vessels, often with localized constrictions, seen in arterial casts are probably produced by contraction of the muscular walls of the arteries associated with the contraction of the capsule and trabeculae of the organ on death, the contraction of the capsule squeezing blood out of the pulp into the venous system and the contraction of the arteries diminishing the inflow.

The arterial capillaries open into the pulp in a funnel-shaped fashion, the endothelial walls of this portion becoming sieve-like and gradually merging with the walls of the pulp spaces. This region would correspond to the ampulla of Thoma (1924), and a rather similar description of it has been given by MacNeal, Otani & Patterson (1927). Neoprene filling the pulp spaces at the ends of the arterial capillaries gives rise to the nodular masses seen in arterial-injected casts.

The extent of development of venous sinusoids varies in different species, giving rise to different appearances in venous neoprene casts (Snook, 1950). In the cat,

neoprene leaves the terminations of large veins in the pulp, giving rise to interconnected, nodular masses of neoprene filling the pulp spaces (Robinson, 1930). The sinusoids are better developed in the sheep and even more so in the dog and human spleens. Neoprene casts of the sinuses in all species examined end in aggregations of small nodules of neoprene filling pulp spaces, due to the continuity of the sinuses with pulp spaces, this being clearly seen in the histological sections. The sinus walls are also in continuity with the reticular tissue walls of the pulp spaces.

Thus, as expected from the embryological development of the organ, capillaries open into pulp spaces and sinusoids lead out of the meshwork of the pulp, the walls of these various components of the circulation all being in continuity.

There is a species difference in the extent of elaboration of the sinusoids into the interior of the pulp from the collecting veins. However, the sinusoids, whatever the extent of their elaboration and degree of specialization of their walls, remain minor modifications of pulp spaces.

The circulation consists of arterial capillaries opening into pulp spaces which are drained by sinusoids which unite until collecting veins are formed. This is fundamentally the circulatory pattern suggested by Klemperer (1938). There may be channels of more direct flow through the pulp from arterial capillaries to venous sinusoids as suggested by Mall (1903) and by Mackenzie, Whipple & Wintersteiner (1941), but clear histological evidence of this is not seen.

In, for example, the dog it would seem that the ellipsoids consist of a persisting, unloosened part of the primitive mesodermal anlage investing the vessels and containing its typical tissue spaces. The intimate relation of venous sinusoids to their periphery suggests that the ellipsoids may act as a short circuit or arteriovenous shunt conveying probably plasma only via their contained tissue spaces, directly from arterial capillary to the investing venous sinusoids, without traversing the pulp proper (Solnitzky, 1937). Blood, separated from some of its plasma, may then enter the pulp through the arterial termination.

The spleen, then, is a part of the reticulo-histiocytic system set in the course of the circulation and possessing arterial channels distributing blood through it and venous channels penetrating it for drainage, so making maximal use of the sponge-like pulp. Blood in its passage through the pulp must be exposed to the activities of the phagocytic cells, and lymphocytes are added to it. In addition, in those spleens possessing them, some plasma may be filtered through the phagocytic cells of the splenic ellipsoids without traversing the pulp proper, though gaining similar treatment.

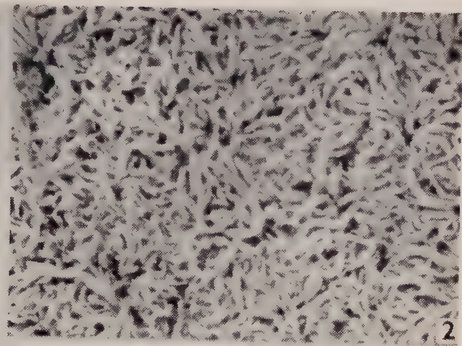
SUMMARY

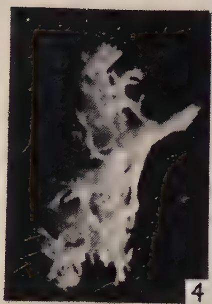
1. The splenic circulation in a number of mammalian species has been studied by a combination of injection and histological methods.

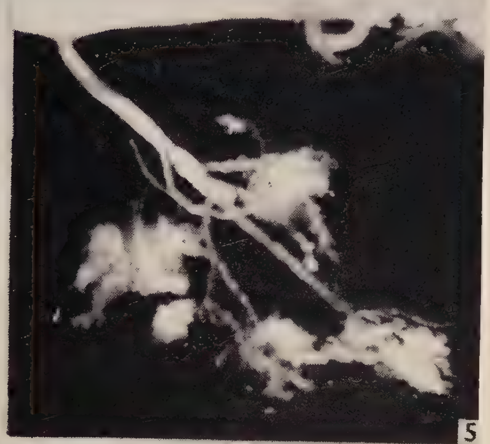
2. In all species examined the arteries are found to form an arborizing pattern unlike the looped system of the embryo.

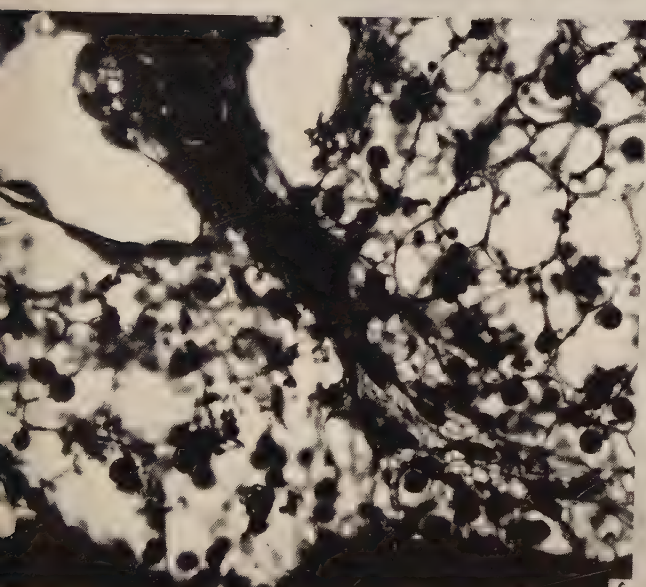
3. Both arterial capillaries and venous sinusoids open into the pulp and the circulation is 'open'.

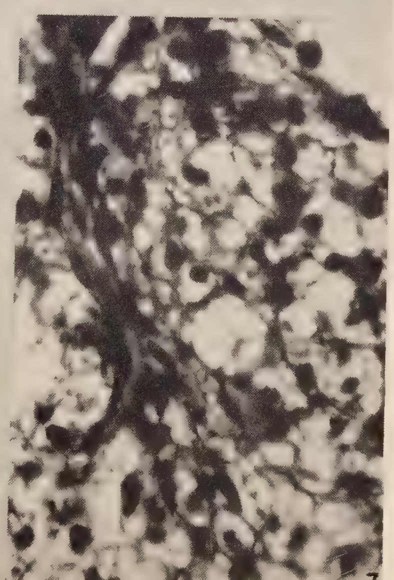
4. The intimate relationship seen in some species between the splenic ellipsoids and venous sinusoids is noted.





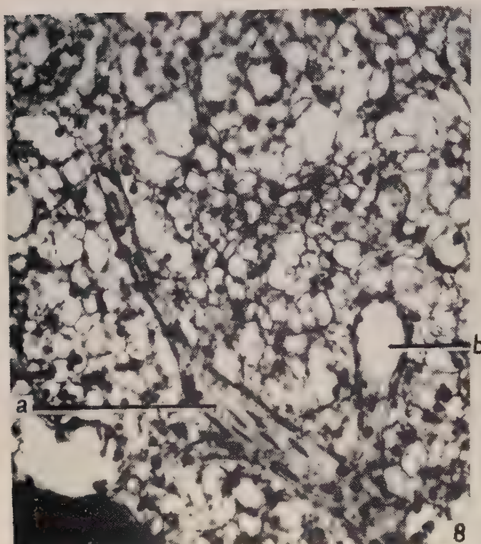


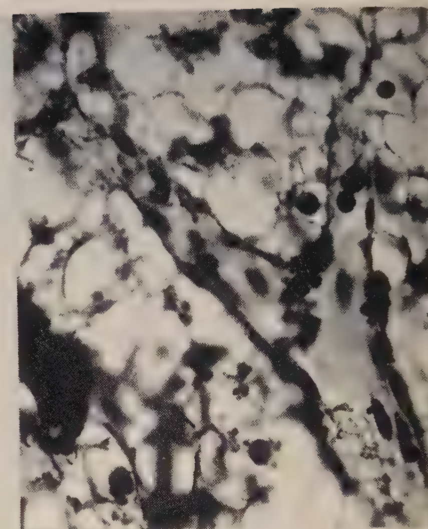


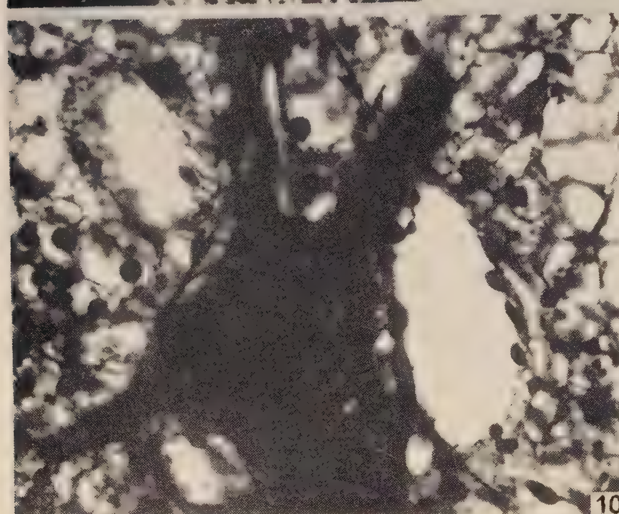


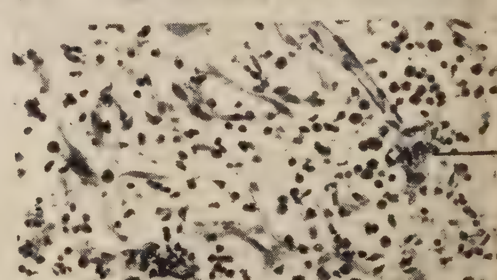
LEWIS—BLOOD VESSELS OF THE ADULT MAMMALIAN SPLEEN

(*Facing p.* 248)









LEWIS—Blood vessels of the adult mammalian spleen

I wish to express my thanks to Prof. D. V. Davies for his valuable advice; to Mr J. S. Fenton and Mr A. E. Clark for the photographs and Mr G. Maxwell for technical assistance.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A portion of a venous-injected neoprene cast of a cat spleen showing the tributaries of a large vein ending in nodular masses of neoprene. ×15.

Fig. 2. Part of a venous-injected neoprene cast of a dog spleen, seen from the surface. A tangled mass of sinusoids, into which the larger veins arborize, take the place of the nodular masses of neoprene seen at the termination of larger veins in the cat spleen. ×20.

Fig. 3. Part of an arterial-injected cast of a sheep spleen showing the spiral form and localized constrictions often seen in the arteries. ×10.

Fig. 4. Part of the interior of the same cast as fig. 2 showing a vein terminating as an arborization of sinusoids.

Fig. 5. Part of arterial-injected cast of a human spleen showing the nodular masses of neoprene at the terminations of the finest arterial branches. ×35.

Fig. 6. A 20μ section of a formalin-distended dog spleen stained with Heidenhain's iron haematoxylin. An arterial capillary can be seen leaving an ellipsoid (invested by its related sinusoids) and opening in funnel-shaped fashion below, its endothelial wall becoming sieve-like and merging with the pulp. ×500.

Fig. 7. The same spleen as fig. 6, showing another capillary opening into the pulp. ×500.

PLATE 2

Fig. 8. The same spleen as fig. 6, showing a sinusoid (*a*) with its lattice walls, commencing in the pulp, its walls blending with the pulp spaces. Another sinusoid (*b*) seen partly in transverse section and partly in surface view is joining it on one side and there is another small tributary joining the other side. ×210.

Fig. 9. The same spleen as fig. 6, showing the origin of another sinusoid from the pulp spaces via two small tributaries. ×500.

Fig. 10. The same spleen as fig. 6, showing an ellipsoid with sinusoids applied to its surface and arterial capillaries leaving it between the sinusoids. ×500.

Fig. 11. An $8\,\mu$ section of a distended dog spleen stained with haematoxylin and eosin. An ellipsoid, consisting of a spongy mass of reticular cells, is shown, with sinusoids applied to its surface and arterial branches leaving or entering it. $\times 210$.

Fig. 12. The same spleen as fig. 6. Part of the wall of a sinusoid can be seen in surface view, its lattice-like form being clearly apparent.

Fig. 13. An $8\,\mu$ section of a distended sheep spleen stained with haematoxylin and eosin. Two of the poorly developed ellipsoids (*a*) can be seen surrounded by the meshwork of the pulp. Strands of smooth muscle can be seen in the pulp. $\times 210$.

OBSERVATIONS ON THE FINE STRUCTURE OF THE PURKINJE FIBRES IN THE VENTRICLES OF THE SHEEP'S HEART*

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INTRODUCTION

The degree of differentiation of the Purkinje fibres, in the ventricles, shows a marked species variation (Holmes, 1921; Blair & Davies, 1935; Truex & Copenhaver, 1947). In the Artiodactyla, the Purkinje fibres are readily recognizable by their large diameter, about four times greater than that of the normal myocardium, by their perinuclear clear space with peripheral cross-striated myofibrils and by their high glycogen content. However, in those species, such as the Carnivora, Rodentia and Primates, in which the fibres are poorly differentiated, none of the above criteria can be used for their identification. In the ventricles of this latter group of species, cardiac muscle fibres with slightly greater diameter and with a small perinuclear clear space may be interpreted as Purkinje fibres or as variations within the normal range of the structure of cardiac muscle. Attention was drawn to this difficulty by Copenhaver & Truex (1952) in their discussion of the presence of Purkinje fibres in the atrial muscle. Davies & Francis (1952) discussed the problem of the nomenclature of these slightly modified fibres, and Blair & Davies (1935) suggested that the term Purkinje fibre should be reserved for the typical fibre, as seen in the Ungulates.

The widely diverse accounts of the distribution of the conducting system in the human heart, ranging from a system disseminated throughout the heart (Todd, 1932) to a denial of its presence (Glomset & Glomset, 1940), have resulted from making subjective interpretations from minor differences in structure.

This electron microscope study was made to establish the fine structure of the Purkinje fibre, in the animal in which it was first described (Purkinje, 1845) and where its identification is undeniable. The possibility was considered that this recent technique might reveal structural modifications which would make the identification of the conducting tissue fibres more definite in other species.

MATERIAL AND METHODS

Three sheep's hearts were obtained from the slaughter house. Specimens less than 1 mm.^{3} were removed and fixed in 1 % osmium tetroxide buffered with bichromate to a pH of 7·6 (Dalton, 1955). The specimens were fixed within 5 min. of the death of the animal.

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In the left ventricles; the left branch of the bundle could be observed grossly under the endocardium of the interventricular septum, and blocks containing the Purkinje fibres and the underlying normal muscle were obtained. In addition, false tendons from the papillary muscles were fixed and subsequently cut into 2 mm. lengths. The moderator bands were removed from the right ventricles and the right branches of the bundle were dissected from the other muscle of the bands before fixation.

After 1 hr. fixation the tissues were rapidly dehydrated in alcohol and embedded in a mixture of butyl and methyl methacrylate. Sections for electron microscopy were cut with a Porter & Blum (1953) microtome equipped with a glass knife. The sections were examined with a RCA EMU2 microscope, without removal of the methacrylate, at initial magnifications of 1000 to 7000 diameters.

Thick sections were cut from the same blocks and were examined by phase microscopy, and after staining with Heidenhain's haematoxylin and with the periodic–acid–Schiff technique (Houck & Dempsey, 1954).

OBSERVATIONS

The Purkinje tisssue was bound into strands, of from one to six parallel rows of fibres, by dense collagenous connective tissue. With phase and electron microscopes, numerous unmyelinated nerve trunks were seen in this surrounding tissue, but in this work nerve fibres were not observed amongst the muscular strands.

Observation of these osmium-fixed methacrylate embedded fibres, by light microscopy, showed that these strands were divided into cells by narrow, irregular clefts (Pl. 1, figs. 1, 3, *I.C.C.*). The roughly cylindrical cells limited by these clefts had a length of about 60μ and a diameter of from 30 to 40μ; as the diameter of the normal cardiac muscle cell, in the same preparations, was about 10μ, the cross-sectional area of the Purkinje cell was about 12 times greater than that of the normal muscle. The electron micrographs demonstrated that the intercellular junctions, bounded by the cell membranes of adjacent cells, were composed of intercellular spaces alternating with areas where the membranes were in close apposition (Pl. 2, fig. 4; Pl. 3, figs. 5, 6; Pl. 4, fig. 8). Where the membranes were in apposition, there were occasional pairs of densely osmiophilic plaques on the sarcoplasmic sides of the cell membranes (Pl. 3, figs. 5, 6). Some of these plaques, especially at the end of the cell, received the termination of the myofibrils, which were always transected by the cell membrane at a point where a *Z.* membrane would have been expected. Many pairs of such dense plaques were seen on the cell membranes unassociated with myofibrillar insertion (Pl. 3, figs. 5, 6).

The cells contained one, or more commonly two, ovoid nuclei usually in the centre of the cell, although occasionally they were seen close to the sarcolemma (Pl. 4, fig. 7) as noted by Blair & Davies (1935). The nuclei contained from one to three nucleoli, composed of densely osmiophilic hollow spheres fused with each other. The outer component of the double nuclear membrane contained the small granules described by Palade (1955).

The intracellular fibrils were present in two distinct groups, one group being arranged as normal myofibrils. The other group were not seen in the normal cardiac muscle and have been named 'Purkinje-fibrils'.

The normal myofibrils were situated almost exclusively in the sarcoplasm close to the cell membrane (Pl. 1, figs. 1–3; Pl. 2, fig. 4). In longitudinal section, these myofibrils, divided into sarcomeres by *Z.* membranes, were composed of myofilaments, which in places showed the characteristic beading of about 400 Å. periodicity. The *I.* bands were small, due to the contracted state and the *A.* bands were hemisected by pale *H.* bands with narrow dark *M.* bands in their centres (Pl. 5, fig. 10). Thus these myofibrils of the Purkinje cell had the same fine structure as those in the normal myocardium, and as has been shown by Weinstein (1954) this is the same as in skeletal muscle. In transverse section, the myofilaments were spaced hexagonally as shown in insect wing muscle by Hodge (1955). As mentioned above, the myofibrils did not pass from cell to cell, but terminated at the cell membrane in dense plaques (Pl. 3, figs. 5, 6). These plaques were visible with the phase microscope (Pl. 1, fig. 1), and were recognizable as the intercalated discs described in the Purkinje fibres of the ox by Jordan & Banks (1917). The cross-sectional area of the myofibrils, in single Purkinje cells, was measured with a planimeter, on low-power ($\times 4000$) electron micrographs. It was found that about 5–15 % of the total cross-sectional area of the cell was myofibrillar, while in the normal muscle the figure was 65–75 %. As the area of the Purkinje cell was some 12 times greater than that of the normal muscle, it follows that although the number of myofibrils per unit volume is diminished, these cells actually contain as many myofibrils as the normal cardiac muscle cell.

The 'Purkinje-fibrils' were found in the perinuclear region and were never seen close to or attached to the cell membranes (Pl. 2, fig. 1; Pl. 4, figs. 8, 9). Although not demonstrated by phase microscopy, they showed as negative images in the PAS preparations and as dark material after staining with Heidenhain's iron haematoxylin (Pl. 1, figs. 1–3). Their fibrillar structure was demonstrated only by electron microscopy (Pl. 4, figs, 8, 9), and it was concluded that they were myofilaments because they demonstrated the 400 Å. periodicity and their diameter was the same as the myofilaments in the ordinary myofibrils (Pl. 5, figs. 10, 11). When the myofilaments in the 'Purkinje-fibrils' were sectioned along their length for a distance equal to a sarcomere no clear banding pattern or *Z.* membranes were seen (Pl. 4, fig. 8; Pl. 5, fig. 11). This observation and the location of these fibres close to the nucleus excluded the possibility that the 'Purkinje-fibrils' were merely normal myofibrils cut in various planes of section. The most common arrangement of the 'Purkinje-fibrils' was in blocks, of sarcomere length, but not oriented to each other and not having *Z.* membranes at the limits of each block (Pl. 4, fig. 8). The myofilaments in each block were not as closely packed as in the normal myofibrils and they did not show the hexagonal arrangement in transverse section. In some of these blocks of 'Purkinje-fibrils' an indistinct dark line, running transverse to the myofilament axis in the middle of each block, was observed and was considered to represent the *M.* band of the normal myofibril; this was the only sign of a banding pattern in these fibrils (Pl. 4, fig. 8; Pl. 5, fig. 11). In addition to the arrangement into blocks, these fibrils were seen as loosely arranged pairs of myofilaments in the perinuclear sarcoplasm (Pl. 4, fig. 9, *P.*).

Glycogen was recognized throughout the sarcoplasm of the Purkinje cells, by its positive PAS reaction and by its pale amorphous granular structure on the electron micrographs. The intracellular localization of the PAS positive material, after

osmium fixation, made it possible to observe negative images of the nuclei, myofibrils and 'Purkinje-fibrils' (Pl. 1, fig. 2).

The ergastoplasm was sparsely distributed throughout the cell, but vesicles carrying Palade (1955) granules could be seen at the sides of the *Z.* membranes of the normal myofibrils (Pl. 5, fig. 10, *E.*). The 'Purkinje-fibrils' did not have any constant relationship to this reticular system.

Mitochondria, similar to those of the normal cardiac muscle cell, were distributed throughout the cytoplasm, but they had a greater concentration in the region of the normal myofibrils (Pl. 2, fig. 4; Pl. 4, fig. 8). The Golgi apparatus was not seen in any of the preparations.

The sarcolemma, with a dark, narrow, inner cell membrane and an outer diffuse layer receiving the attachment of collagen fibres, was identical with the sarcolemma of normal myocardium (Pl. 4, fig. 9).

DISCUSSION

In their early electron microscope studies, Kisch, Grey & Kelsch (1948), in the human heart, and Beams, Evans, Janney & Baker (1949), in the guinea-pig, thought that they had seen 'conductive fibres'. As identification of the conductive tissue in these species is difficult, and as the observations in the present work, on the clearly defined Purkinje fibres of the sheep, do not resemble their micrographs, it is not possible to correlate this report with earlier work with the electron microscope.

The cellular organization of the Purkinje tissue was described and illustrated by Tawara (1906), who likened these cells to the stratum spinosum of the epidermis. The accuracy of this observation is vindicated by a comparison of the electron micrographs in the present work with recent work on the skin (Selby, 1955). On the basis of maceration experiments, Tufts (1921) concluded that the Purkinje fibres were cellular, but most authors since Tawara (DeWitt, 1909; Blair & Davies, 1935; Field, 1951) have supported the syncytial concept. In the Purkinje fibres of the ox, Jordan & Banks (1917), strongly supporting the syncytial theory, admitted that embryonically these fibres are cellular and that in the adult fibre the fused sarcolemmae can be seen. This present demonstration of the cellular structure of Purkinje fibres agrees with the conclusions of Van Breeman (1953), Sjöstrand & Andersson (1954), Price, Weiss, Hata & Smith (1955) and Muir (in press) that normal cardiac muscle is cellular.

The Purkinje cells are shorter than the normal cardiac muscle cells and hence have more intercellular junctions (intercalated discs) for a given length of fibre. This observation raises the problem of transmission of the impulse for contraction across these intercellular junctions. Lewis & Rothschild (1915) stated that, in the dog, the conduction rate in the Purkinje tissue is nine times faster than in the normal myocardium. If this, generally accepted, statement is true, then either there must be insignificant delay at each junction, or the conduction rate within each cell is very rapid indeed. The present demonstration of numbers of structures on the cell membranes, similar to intercalated discs but not receiving the insertion of myofibrils, may be related to the spread of contraction from one cell to the next, both longitudinally and transversely, in the Purkinje strand.

This electron microscope study shows little structural resemblance between the Purkinje cell and embryonic myocardium (Muir, in press), apart from the simple form of intercalated disc and peripheral distribution of myofibrils. The short, broad, cylindrical Purkinje cell often with two nuclei contrasts sharply with the long, spindle-shaped, uninucleated embryonic cardiac muscle cell. The statement that the Purkinje cell fails to acquire its full complement of myofibrils (Field, 1951) would appear to be erroneous, as the present work indicates that the number per cell is at least approximately the same as in the normal cardiac muscle cell. This latter observation supports the findings of Truex & Copenhaver (1947) and Muir (1954) who illustrate the continuity of myofibrils at the region of transition from the Purkinje to the normal cell.

Recently, Carbonell (1956) has demonstrated that conductive tissue fibres contain a higher concentration of cholinesterase than normal cardiac muscle. In the Ungulates, he demonstrated that the esterase was confined to the periphery of the cell and concluded, on this basis, that it was localized in the myofibrils. The present demonstration that most of the mitochondria are adjacent to the myofibrils would suggest a possible alternative localization of the enzyme.

Tawara (1906) and Field (1951) have referred to the granular material in the perinuclear cytoplasm, and Marceau (1902) noted that these granules stained intensely with iron haematoxylin, while Aschoff (1908) considered that they were composed of glycogen. The present work shows, however, that these granules are PAS negative and that they have a myofilamentous structure. The lack of orientation of these fibrils and their limitation to the central part of the cell would make it unlikely that they contribute any force of contraction. The relative absence of mitochondria and ergastoplasm near these 'Purkinje-fibrils' would suggest that this is not a functionally active part of the cell. Ashley, Porter, Philpott & Haas (1951) have shown that trypsin digestion caused myofibrils from skeletal muscle to separate into sarcomeres at their *Z.* membranes; thus their preparations closely resemble the blocks of 'Purkinje-fibrils'. The possibility that these fibrils represent normal myofibrils which have been separated, at their *Z.* membranes into sarcomeres, by the accumulation of glycogen and that they remain in the central sarcoplasm as inactive remnants must be considered. Studies on other parts of the conduction system, on other species, and at various stages of development should provide evidence as to their possible function.

SUMMARY

Purkinje fibres from the ventricles of adult sheep's hearts have been examined by light and electron microscopy, after osmium fixation and methacrylate embedding.

The observations which were made showed that:

1. The Purkinje fibres consist of separate, short, cylindrical cells, without continuity of the myofibrils at the cell junctions.
2. Simple forms of intercalated discs transect the myofibrils at the cell junctions.
3. The myofilaments are arranged in two groups, as normal myofibrils at the periphery of the cell, and as 'Purkinje-fibrils' in the perinuclear sarcoplasm.
4. The myofibrils in the Purkinje cells have the same structure as in the normal

cardiac muscle cell. There seem to be about as many myofibrils in a Purkinje cell as in an ordinary muscle cell, but the larger size of the former makes the number per unit volume less in these cells.

5. The 'Purkinje-fibrils' consist of myofilaments, either loosely scattered in the sarcoplasm or arranged in blocks with a length equal to that of a normal sarcomere. The 'Purkinje-fibrils' do not exhibit any *Z*. membranes or well-marked banding pattern.

The author wishes to thank Dr E. W. Dempsey for providing the necessary facilities, and for much helpful criticism and advice.

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EXPLANATION OF PLATES

All the figures are of Purkinje fibres from the ventricles of the sheep's heart, which were fixed in buffered osmium tetroxide and embedded in methacrylate. Pl. 1: light microscopy; Pls. 2–5: electron microscopy.

A.	*A.* band	*M.B.*	*M.* band
D.	Intercalated disc	*M.I.*	Mitochondrion
E.	Ergastoplasm	*N.*	Nucleus
I.	*I.* band	*P.*	'Purkinje-fibril'
I.C.C.	Intercellular cleft	*S.*	Sarcolemma
M.	Myofibril	*Z.*	*Z.* (Krause's) membrane

Plate 1

Fig. 1. Phase contrast: the cross-striated myofibrils are distributed mainly in the peripheral cytoplasm of each cell. Intercellular clefts can be distinguished, but it is not possible to resolve the cell membranes where they are in apposition. The intercalated discs are seen as dark bands on the myofibrils, in series with the Z. membranes. There is a large, clear, perinuclear region. ×1350.

Fig. 2. Periodic-acid–Schiff reaction: the glycogen is distributed evenly throughout the sarcoplasm. The myofibrils, nuclei and parts of the perinuclear region are seen as negative images. ×470.

Fig. 3. Heidenhain's iron haematoxylin: in the perinuclear region, material (*P.*) which was not evident on phase microscopy, and which showed a negative PAS reaction, is seen to be stained. Intercellular clefts can be seen. ×1100.

Plate 2

Fig. 4. One Purkinje cell, bounded on the right by the sarcolemma, is illustrated. The cell membranes limiting the cell above and to the left are indicated by arrows. The amorphous, granular cytoplasm is consistent with a high glycogen content. The myofibrils and, to a lesser extent, the mitochondria are distributed in the peripheral cytoplasm leaving a relatively clear zone around the nucleus. In this clear zone, 'Purkinje–fibrils' (*P.*) showing no orientation relative to the axis of the cell can be seen. ×4600.

PLATE 3

Fig. 5 The cell membranes separating the upper from the lower cell can be traced, above the arrows, to the sarcolemma on the right. The membranes are, in places, separated leaving intercellular clefts. Where the membranes are in apposition, there are occasional osmiophilic plaques (intercalated discs) receiving the termination of myofibrils. $\times$ 13,500.

Fig. 6. Portions of three cells can be seen, their separating cell membranes meet at the point of the arrow. The myofibril in the lower right cell ends at the disc, which is situated where the next *Z.* membrane would have occurred. The transversely sectioned myofibril exhibits, in the circle, the hexagonal arrangement of myofibrils. $\times$ 13,500.

PLATE 4

Fig. 7. The nucleus, which is situated unusually near the sarcolemma, is seen to be limited by a double membrane. The outer membrane contains large numbers of small, dark, round granules. $\times$ 17,500.

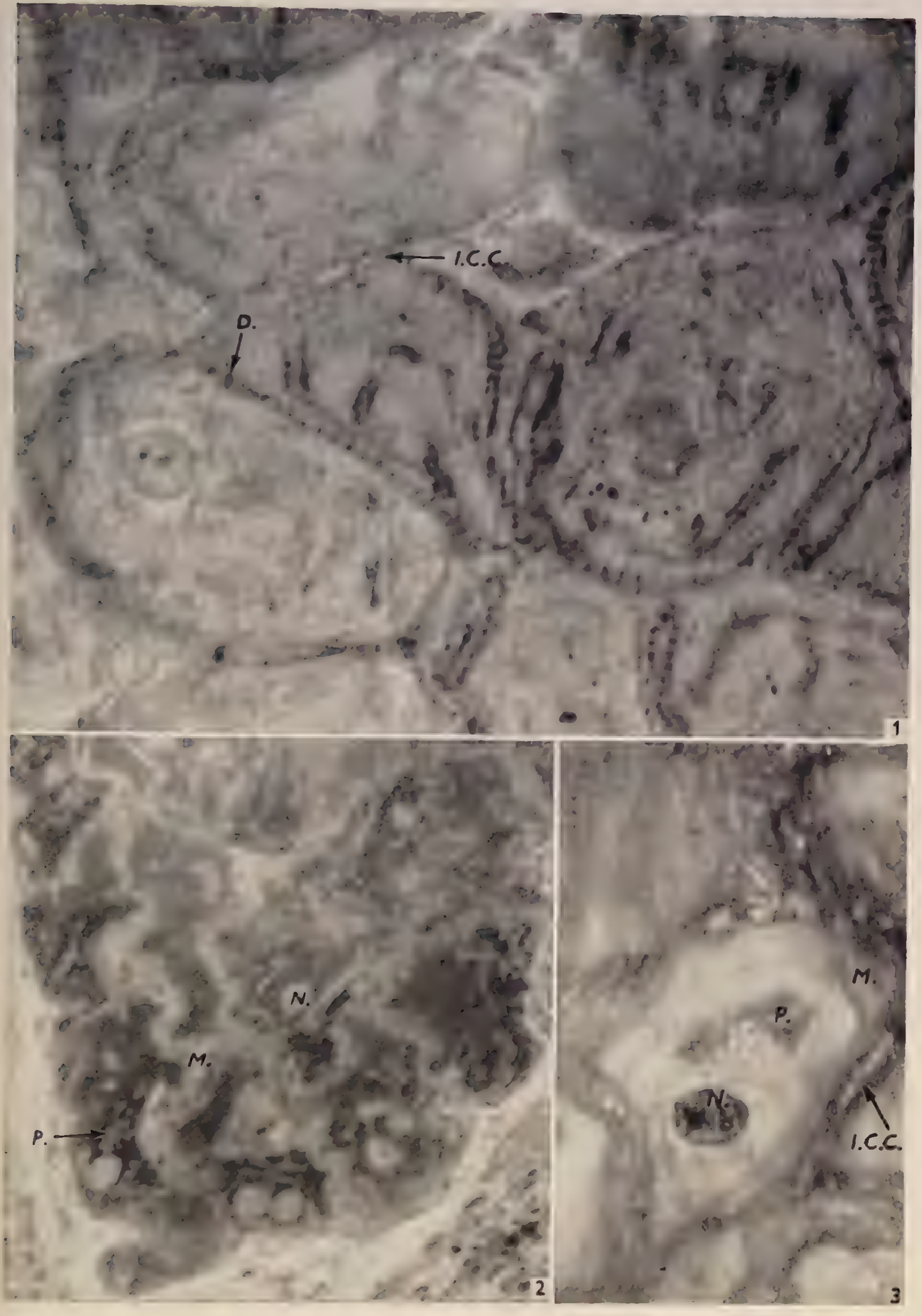
Fig. 8. A pair of cell membranes, separated in places, runs obliquely across the field between the two arrows. Ordinary myofibrils, with *Z.* membranes, are seen in each cell close to the cell membranes. While in the central cytoplasm of each cell, blocks of 'Purkinje-fibrils' (*P.*) are evident. Most of these blocks are sectioned along the length of their component fibrils, yet no *Z.* membranes can be seen. In the myofibril, as at the tip of the upper arrow, the *M.* band can be seen clearly, and in one of the blocks of 'Purkinje-fibrils' a faint indication of an *M.* band (*M.B.*) can be made out. The mitochondria are chiefly distributed near the myofibrils, with fewer in the region of the 'Purkinje-fibrils'. $\times$ 7200.

Fig. 9. A higher magnification of part of the cell shown in Fig. 4: the sarcolemma shows an inner, narrow dark membrane and an outer, thicker, diffuse layer of less density. Collagen fibres, sectioned transversely, are seen outside the sarcolemma. Under the sarcolemma, there is a normal myofibril and at the left of the picture there are loosely arranged myofilaments forming 'Purkinje-fibrils'. On a pair of these myofilaments, between the arrows, a beading with a periodicity of 400 Å. can be discerned. $\times$ 26,500.

PLATE 5

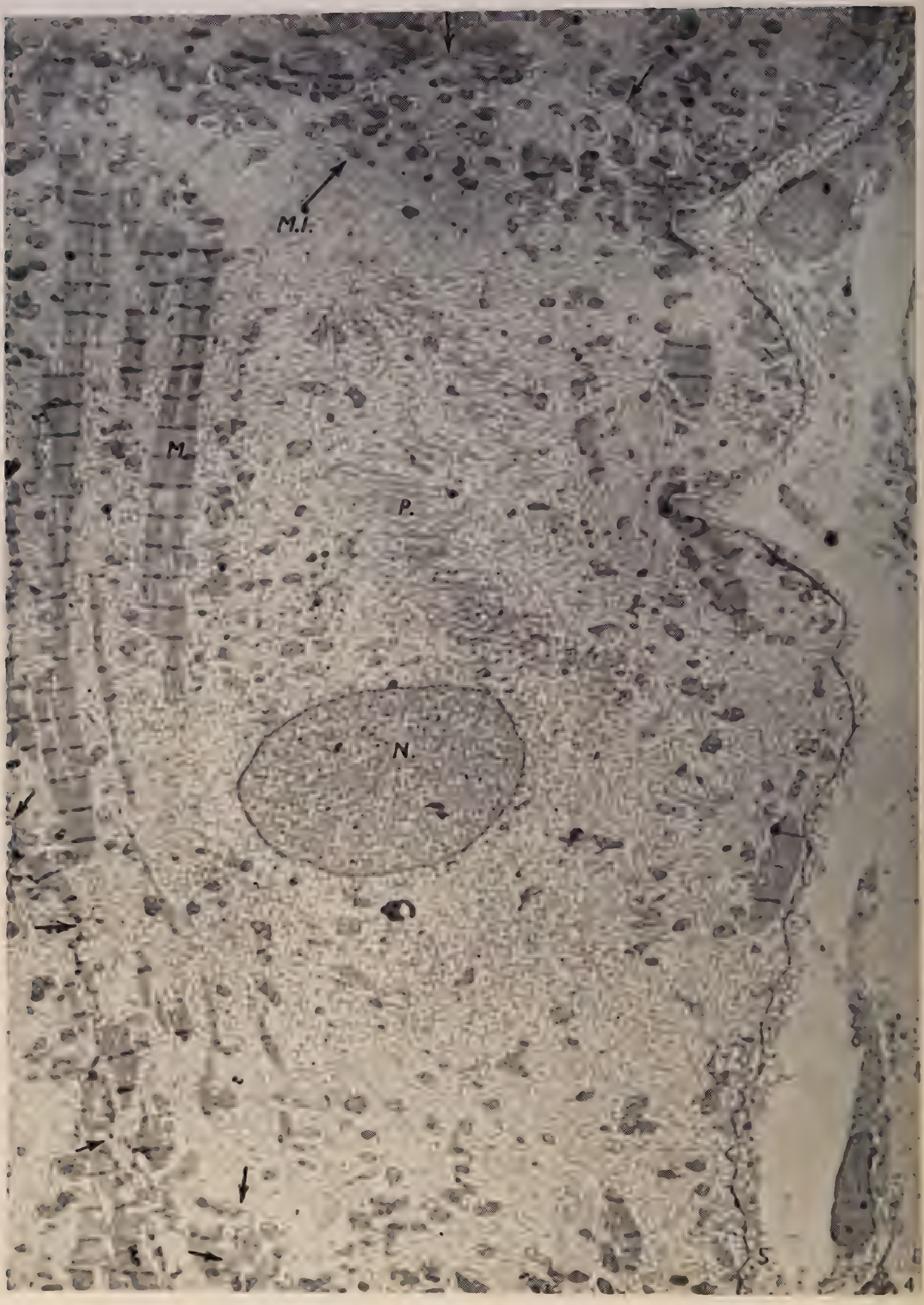
Fig. 10. The normal myofibrils are seen to demonstrate a complete banding pattern. The myofilaments can be traced through the dense material forming the *Z.* membranes. Parts of the ergastoplasmic network can be seen in association with the *Z.* membranes. $\times$ 27,600.

Fig. 11. 'Purkinje-fibrils', from the centre of a cell, are illustrated at a similar magnification to that of the normal myofibrils above. The length of each block of fibrils is about equal to the length of a normal sarcomere. These myofibrils do not exhibit any *Z.* membranes or clear banding pattern. Between the arrows there is a slight increase in density which may represent an *M.* band. $\times$ 22,000.

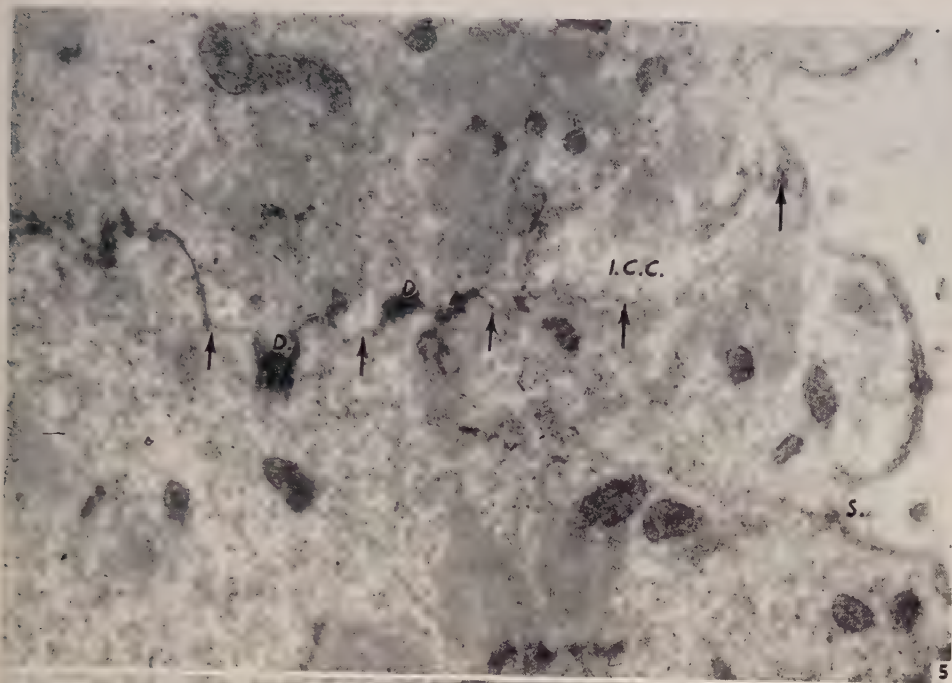


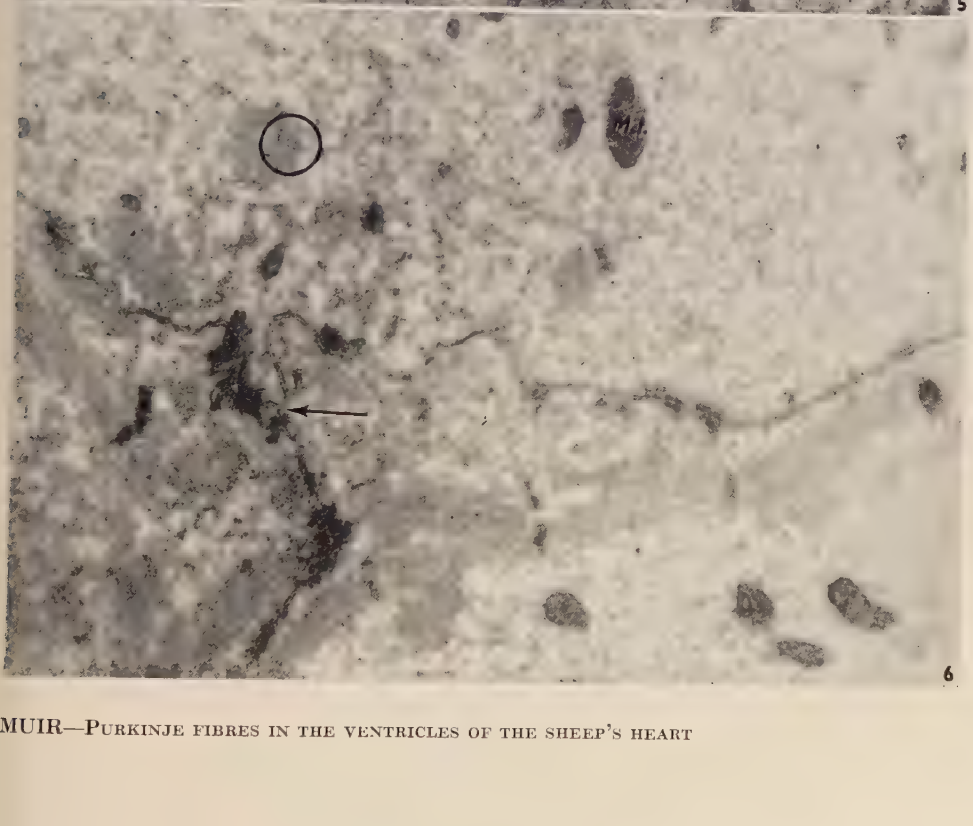
MUIR—Purkinje fibres in the ventricles of the sheep's heart

(*Facing p.* 258)



MUIR—PURKINJE FIBRES IN THE VENTRICLES OF THE SHEEP'S HEART





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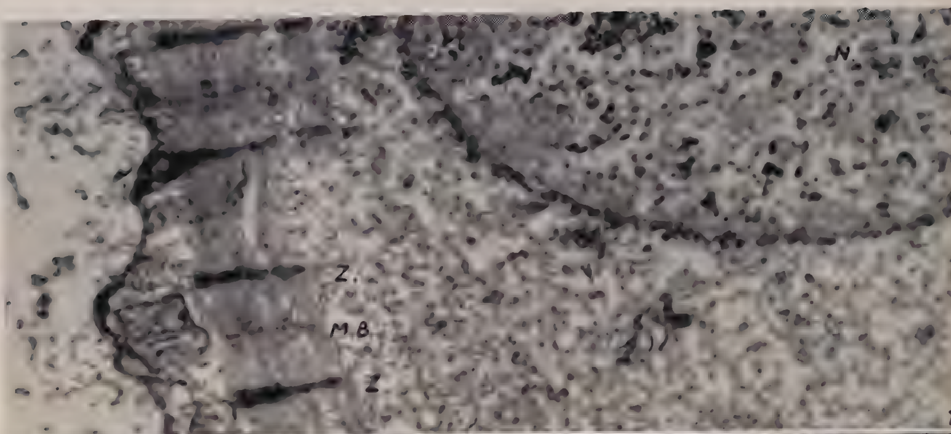
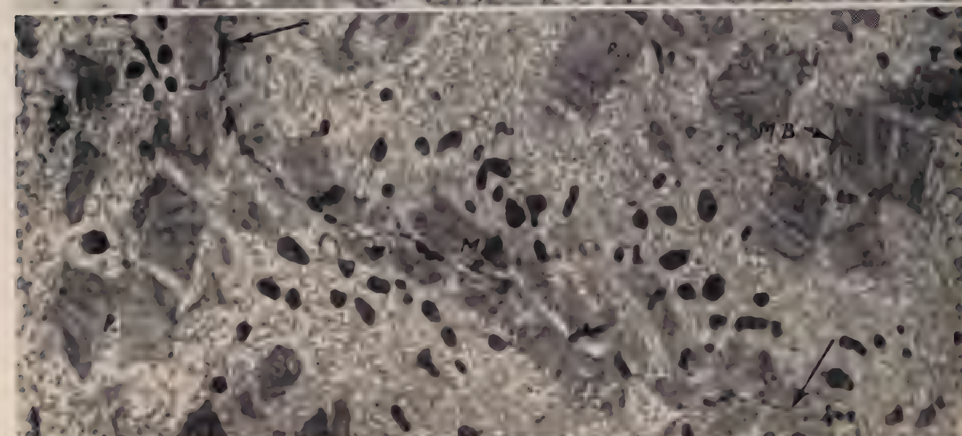
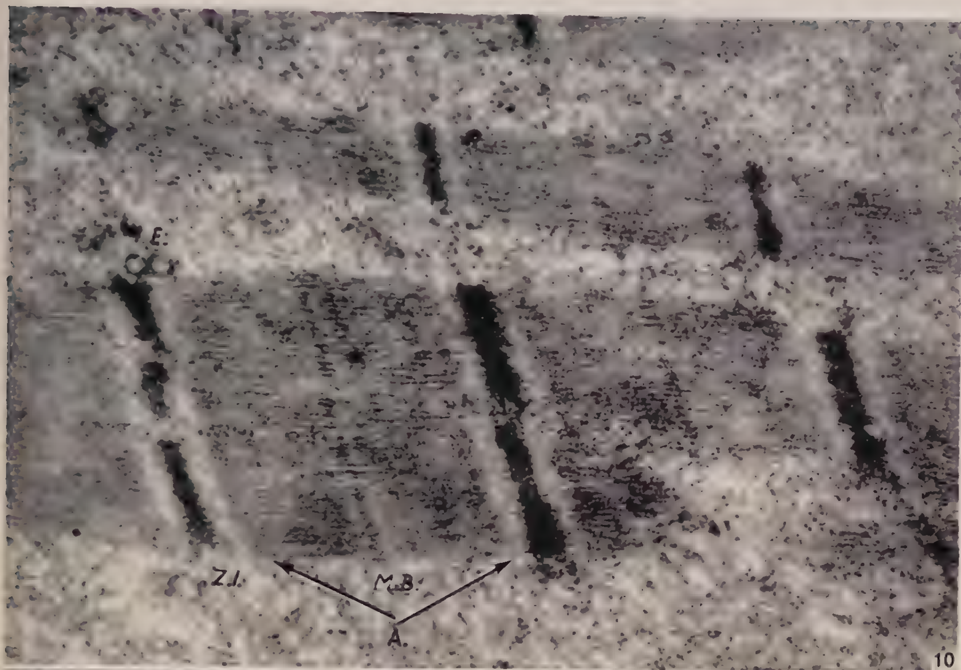
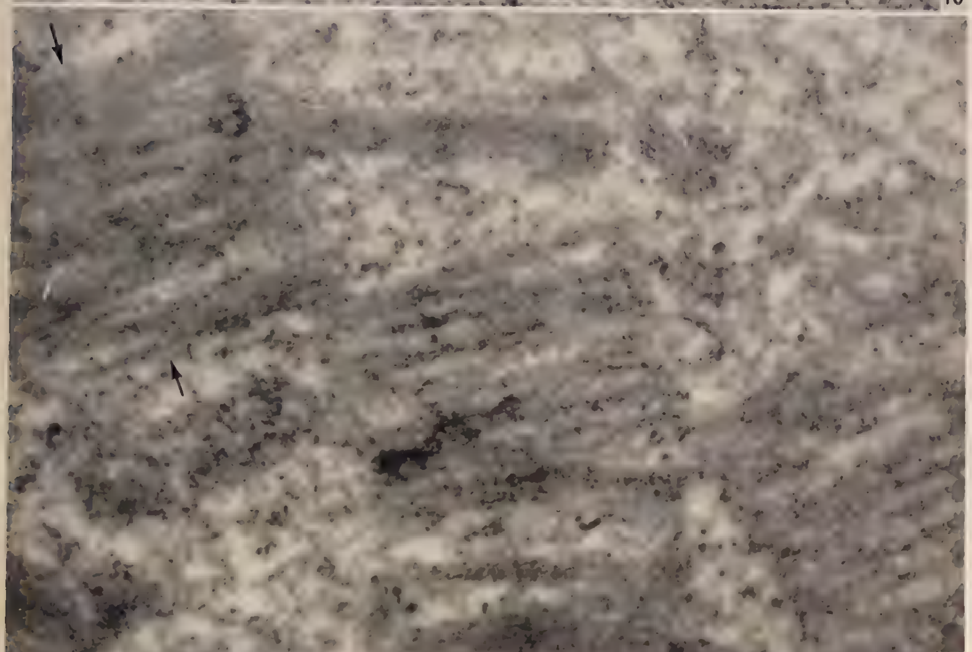




MUIR—Purkinje fibres in the ventricles of the sheep's heart





MUIR—PURKINJE FIBRES IN THE VENTRICLES OF THE SHEEP'S HEART

STRUCTURES IN THE ATRIAL ENDOCARDIUM OF THE DOG WHICH STAIN WITH METHYLENE BLUE, AND THE EFFECTS OF UNILATERAL VAGOTOMY

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INTRODUCTION

Two main types of nervous structure in the atrial endocardium have been described, namely a syncytial network of fibres and cells, and free endings. Recent workers such as Meyling (1953) and Mitchell (1956) have doubted that discrete endings exist in this site, although earlier workers employing methylene blue staining methods had observed both types of structure. Thus Smirnow (1895) described a nervous network from which arose free endings; Nettleship (1936) demonstrated similar structures in the cat's heart, and also nerve endings apparently unconnected with the net; while Woollard (1926) had noted similar nervous formations in the heart of the dog. Dogiel (1898) figured nerve endings very clearly.

Workers employing silver impregnation techniques, and examining sectioned material, have usually described only free branched terminations of nerve fibres. Nonidez (1937, 1941) described such structures in young cats and dogs; Pannier (1940) found them in the endocardium of cat atria, and Sato (1954) and Tcheng (1951) in the dog. Recently the present writer correlated the appearance of such nervous structures in the atrial wall when prepared by methylene-blue and silver methods (Holmes, 1956); and as a result of combined physiological and histological investigations, evidence suggesting that branched terminations of thick endocardial nerve fibres are truly receptor in function has been put forward (Coleridge, Hemingway, Holmes & Linden, 1957). Nettleship (1936) showed that endocardial nerve endings disappeared following bilateral vagotomy below the nodose ganglia, but not above. He concluded that they were sensory in nature. He also found that the finer nerve fibres forming the atrial endocardial plexuses degenerated following infra-ganglionic section. Previously Smirnow (1895) had described the results of vagal section on one side in the cat, with section also of the contralateral depressor nerve. He found, as Nettleship later confirmed, that the large endings disappeared, but stated that the subendocardial nerve net remained intact. Woollard (1926) found no effect on either structure after bilateral removal of the stellate ganglia.

MATERIAL AND METHODS

Adult dogs were used in the present work. The atria of four normal animals were examined after staining with methylene blue by Mitchell's method (1953). Following immersion overnight in saturated ammonium picrate solution, partial clearing was obtained by soaking for some hours in equal parts of glycerine and picrate; areas of atrial wall were then removed, and after trimming off enough of the muscle

layer to allow adequate light penetration, the remaining tissue, with the endocardial surface uppermost, was mounted in glycerine jelly saturated with ammonium picrate. The whole extent of the atrial surface was examined in each animal.

In four other animals a unilateral section of the combined vago-sympathetic trunk was made in the upper cervical region 1·5–2 cm. below the level of the nodose ganglion, about 1 cm. of trunk being removed. The animals were allowed to survive 7, 9, 12 and 44 days respectively, and the whole endocardium then examined after being treated in the same way as in the control animals.

OBSERVATIONS

The structures in the atrial endocardium which stain with methylene blue are several, but nervous elements are the most prominent. In certain areas thick myelinated fibres are present. These enter the endocardial layer from the myocardium and run for considerable distances in the connective tissue, usually taking a sinuous course and branching (pl. 1, figs. 1, 2). They show nodes of Ranvier, and typically, intense staining for a short distance on either side of the node. The diameter of these fibres may reach 10μ, and occasional fibres up to 14μ have been observed.

At their ends discrete terminal structures are formed. The thick fibre divides into finer branches; the myelin sheath is lost; and fine filaments fan out in the thickness of the endocardium (Pl. 1, fig. 3). These finest threads are closely associated with elements which often show a deeply stained peripheral rim surrounding a lightly stained central region; while along their course are numerous small varicosities. The size of the whole structure varies, the larger ones from the thickest fibres reaching 350μ in greatest diameter in the plane of the endocardial surface, although smaller ones of about 200μ are more common; they also extend in depth throughout a considerable distance. A few fibres form only a single ending. Most, however, in addition to branches given off in their course, divide several times near their terminations, and each division runs to an end formation; there is thus a tendency for them to occur in groups originating from branches of a single fibre (Pl. 1, fig. 1).

The distribution of these end formations is limited. They are found particularly in the junctional regions where the venae cavae and pulmonary veins enter the atria, in the intervenous regions of the atrial wall, and over part of the interatrial septum. They have not been found in the endocardium of the auricular appendages nor around the attachments of A–V valves. The total number in the whole atrial endocardium varies, but may be of the order of 150, arising from one-half to one-third of that number of fibres. It is impossible to give precise figures, for some fibres branch so frequently near their terminations that it is often difficult to decide when to distinguish between a number of closely lying small terminal structures and a large diffuse one. The distribution ratio between the right and left atria also shows considerable variations in different animals, although it is probably correct to say that the right atrium receives somewhat less than half the total, the left more. In one normal animal, however, the ratio left : right was 4 : 1.

Fibres of smaller calibre usually run to smaller endings. In some zones, several

of these smaller myelinated fibres form by repeated branching a complex network, from which fine terminal branches arise (Pl. 2, fig. 7); and in these regions, although discrete endings may be seen, the whole complex often appears rather to form a 'terminal area' which, in spite of its net-like appearance, is distinguished from the more generally distributed network of fibres described below.

The second structure demonstrated in the endocardium is a network of nerve fibres and associated cells, forming a syncytium (Pl. 1, fig. 4). Described in various organs (see Meyling, 1953; Mitchell, 1956), this has been called the 'terminal nervous network'. It is of more general distribution than the discrete endings described above, being found over most of the atrial endocardium and extending into the ventricles. The appearance of the net varies considerably even within relatively small areas. In some regions, particularly the intercaval endocardium, near the bases of the A–V valves and in the auricular appendages, thick strands composed of a number of fibres are commonly seen (Pl. 1, fig. 5) as well as single filaments of the network. The fine filaments appear beaded, due to small masses of darkly staining material which lie along their length (Pl. 1, fig. 6).

Thicker fibres, apparently finely myelinated, can be observed giving off fine branches into the general network, and after repeated branching losing their identity within it. These fibres do not reach the size of the largest fibres which run to discrete endings.

It is exceedingly difficult to be sure of the relationship between the large terminal structures and the fine nervous network. The net often co-exists with the large endings, and can be seen passing over the latter, lying nearer to the endocardial surface, without communication. Occasionally, however, fine nerve threads can be seen to leave thick fibres, or to run from their terminal divisions, and pass into the net. Close examination in favourable areas shows that these communications may sometimes be more apparent than real. Fibres of the net occasionally lie very close to a thick fibre for some distance; and it may be found that an apparent branch of this thick fibre can also be traced into continuity with the net further back, so that thin and thick elements merely lie in contiguity over a part of their course. Such contiguity may also occur in the region of the terminal branching of the thick fibre; but here the staining of fibres and associated elements is usually so intense that while an overlying net may be clearly shown, high-power examination in the plane of the ending is not conclusive.

It must be emphasized that the 'terminal nervous network' or general net just described appears to differ in structure from those zones where a medullated fibre branches repeatedly to form a diffuse type of end formation, which has been called a 'terminal area'. These two elements may co-exist in parts of the endocardium; but while the meshes of the general net vary in size, they remain meshes. Those formed in the field of a diffuse ending, however, appear as a pattern of dividing fibres, from which terminal branches arise.

True ganglion cells, as opposed to the 'autonomic interstitial cells' of the terminal network, are rarely found in the endocardial tissue, although they are common in epi- and myocardial layers. Occasional small groups have been observed, lying in the plane of the nervous network. The form of these cells is usually bipolar, but occasional apparently unipolar cells have been seen, the single process dividing a

short distance from the cell body. The processes of these cells pass into the nervous network (Pl. 2, fig. 8).

In addition to the nervous structures described above, another endocardial element has been constantly demonstrated in the present series of preparations. This takes the form of thick, deeply staining fibres which extend into the endocardium from the myocardial coat, and which in suitable fields can be traced into continuity with muscle fibres of the myocardium. They may be disposed singly, or in groups showing a syncytial structure (Pl. 2, fig. 9), especially in the intercaval part of the right atrium, at the bases of the A–V valves, and in both auricular appendages. The surface of these fibres is often studded with deeply staining globular bodies, of varying size, which may be so numerous so as to obscure their internal structure. In some of the lighter stained examples, however, nuclei can be discerned, together with tranverse striations and intercalated discs. As they are traced deeply towards the muscle coat, these specific staining characteristics disappear.

The ends of these fibres come to lie in the plane of the endocardial nerve network, and nerve filaments lie very close, or in contact, with them (Pl. 2, fig. 10). In several instances it appeared as if the tapering end of a muscle fibre actually continued into the nervous network, although this appearance could be a result of close contact between the two structures.

Muscle fibres running into the endocardial connective tissue had previously been noted in a series of silver-stained sections of atrial wall; confirmation of the identity of those fibres which were seen in methylene-blue preparations was sought by sectioning and staining a small area of atrial wall which had originally been stained by methylene blue, and which had shown these structures. These sections showed scattered fibres entering the endocardium from the myocardium, and penetrating halfway or more to the endocardial surface. Sections stained with haematoxylin and eosin, iron haematoxylin or Masson's connective tissue stain, as well as those impregnated with silver, showed no structural differences between these fibres and those of the main muscle layers.

Preparations stained with methylene blue usually also demonstrate varying numbers of fibroblasts lying in the endocardium.

OBSERVATIONS FOLLOWING OPERATION

The main object of the partial denervation carried out by section of one vago-sympathetic trunk was to observe degeneration of the thick myelinated fibres and their endings, and to attempt to elucidate the relationship between these and the general nerve net.

Three animals were subjected to a right trunk section, and examination of the atria made after 7, 12 and 44 days survival. The fourth animal was subjected to a left section, and allowed to survive 9 days.

In those animals examined 7, 9 and 12 days after operation, occasional degenerated large fibres and endings were found, not limited to the atrium on the denervated side. The signs of degeneration were widening of the nodes, with diminution or loss of nodal staining; while the terminal structures appeared either as a group of darkly

staining but separated elements of irregular shape (Pl. 2, fig. 11), or were deformed and pale staining. Normal endings, showing full deep staining, could be found close to degenerate ones, in one case within 0·3 mm. (Pl. 2, fig. 12).

Normal variations in net structure and distribution are so great that it was not possible to be certain whether the nerve section had caused any diminution or not; but certainly there was no marked disappearance. Well stained and apparently normal net fibres were observed close to degenerated endings, i.e. within the same low-power field, or even overlying them. Absence of a network over a degenerated ending is not necessarily indicative of its disappearance, for many normal endings are not closely associated with net fibres. As for the relation between the large terminal structures and the net, only one preparation was found which assisted in the elucidation of this. In the animal which survived 12 days, a large, pale staining and distorted ending was found, with a normal nerve mesh lying within 50 μ, and a fine fibre overlying the degenerate ending. From one of the faintly stained distal parts of the end formation, a fibre appeared to run and enter into the network. This fibre, from the point where it was seen leaving the distorted mass of the ending, appeared normal. The 44-day animal showed only normal endings; the period since operation in this case was great enough for all traces of degenerate endings to disappear. Both sides of the heart showed good nerve nets.

An attempt was made to assess the effect of the partial denervation on the atria by counting normal end structures remaining in the whole left and right endocardium, but difficulties arose owing to the necessarily arbitrary definition of a 'single' ending previously referred to. Combined totals for both atria varied from 89 to 175, the latter figure exceeding that found in any of the four control animals. In all the operated animals the left atrium showed three or four times the number of normal end structures found in the right; the most surprising figures were those for the dog subjected to a *left* nerve section, in which numbers for the left and right atria were 93 and 16 respectively. These figures show wide variations and indicate that the effects of a partial denervation do not readily lend themselves to numerical treatment.

DISCUSSION

Two types of nervous structure can be shown to exist in the atrial endocardium; namely, the complex terminations of thick nerve fibres, and a more widely distributed fine fibred endocardial network. The large terminations can be correlated with endings described in silver impregnated sections by Nonidez (1937, 1941) and many other investigators. A study of a large number of such structures has shown that they are predominantly 'terminal' and not merely points of entry of thick fibres into the general net, although some fine communications would appear to be present. Comparison of these terminal zones with those areas where medium or fine fibres do enter the general network shows two very different patterns. In the latter sites, the fibres can be followed, repeatedly dividing, until they blend with the meshes of the net; whereas in the former, the thick and deeply staining fibres come to an abrupt end. If this was a point where they joined the net, and if this was their sole purpose, one would expect to see more than the occasional small and often ill-defined filaments extending between the two structures; for even

when well-stained meshes actually overlie the terminal structures, communications are rarely apparent.

'Terminal areas' such as that shown in Pl. 2, fig. 7, are probably variants of the typical end formations, related especially to the terminations of finer fibres, but likely to have similar functions. These 'netlike endings' differ structurally, and presumably may differ functionally, from the regular networks such as shown in Pl. 1, figs. 4 and 5.

Typical end formations such as those described and illustrated are not exclusive to the heart wall. Larsell (1921) figures similar nerve endings in rabbit lung stained by methylene blue. He did not consider the swellings associated with the terminal branches to be cellular in nature, since he failed to demonstrate nuclei. Dogiel (1898), on the other hand, thought that the swellings themselves were nuclei of syncytial 'star cells', whose cytoplasmic processes formed a scaffold for the fine branching nerve filaments. The present writer originally considered them to be cellular in nature, but close examination of numerous end formations has failed to reveal any typical nuclear structure in these swellings, whether stained by methylene-blue or silver techniques. They show wide variation in size and density of distribution in various endings and it is possible that they form a more uniform covering for the fine fibres in the living state. Degenerating end formations show no distinctive cellular characteristics after methylene-blue staining.

Partial denervation of the heart indicates great variability in the atrial nerve supply from the vagus. Following unilateral vagotomy the thick fibres and their endings do degenerate, and some can be seen within the first 2 weeks, distorted and in process of disintegration, but still recognizable; others have doubtless already lost their staining properties within a week of section. The persistence of normal endings very close to degenerate ones is probably indicative of considerable intermingling of fibres from the right and left vagi over the atria, although it must be noted that in the present series of animals the superior laryngeal nerve was not cut, and therefore remained as a possible source of a few cardiac fibres. As for the network, a study of partially denervated atria does not enable any firm conclusions to be drawn, although the indications are that it does not degenerate after damage to extrinsic nerve fibres. Nettleship (1936), reporting that the network disappeared in the cat following bilateral infra-ganglionic vagotomy, may perhaps have been describing the disappearance of the 'netlike endings', which will degenerate in the same way as the larger and more clearly defined terminal structures.

If, as has been suggested (see review by Meyling, 1953), the terminal nervous network is truly a syncytium of fibres and 'autonomic interstitial cells', with which extrinsic nerve fibres make synaptic contact at various points, degeneration would not necessarily be expected to pass into the network following section of extrinsic nerves, even if these were supplying the net in some way.

As regards the function of these various structures, evidence has been offered (Coleridge *et al.* 1957) that the discrete endings are receptor in nature. The grounds for this conclusion were the demonstration of typical branched end formations in silver impregnated sections taken from physiologically localized receptor areas. Such structures are essentially the same as those demonstrated in methylene-blue preparations. It is a matter of debate whether the terminal nervous network could

also be receptor, although Meyling suggests that it could be both sensory and motor. It is difficult to envisage a widespread sensory network allowing the localization of a small 'receptor area'. Such areas were found physiologically, and nerve impulses recorded from functionally 'single vagal fibre' preparations on mechanical stimulation within a small and localized region, 1–2 mm. in extent. This is an area comparable to that occupied by a single branching vagal fibre and its terminal structures. It may be noted that some form of network has been demonstrated in many different organs (Meyling, 1953; Mitchell, 1956).

The constant presence of myocardial fibres in parts of the endocardium, their typical staining properties in this site, and their close relationship to fibres of the terminal nervous network has been noted. Blair & Davies (1935) described the presence of similar fibres in the bovine heart, and illustrated them clearly in figure 8 of their paper. These fibres do not form a general syncytium over the whole extent of the endocardium, but occur as small syncytial groups, or isolated fibres. They are not 'Purkinje fibres' in the usually accepted sense, neither are they continuous with any part of the conducting system as we accept it. But it seems possible that these fibres, and the closely related nerve fibres, might play some part in the process of conduction of the contractile impulse through the atrium.

The appearance of fibroblasts in methylene-blue preparations was ascribed to excess staining by Nettleship (1936); but they were seen even with the weak concentrations of dye used in the present study, and are probably a normal finding.

SUMMARY

The endocardium of the dog atrial wall after methylene-blue staining shows thick myelinated fibres which run to circumscribed end formations. These are located particularly about the points of entry of the veins, and are thought to be receptor in function. A second nervous structure consists of a more widely distributed fine fibred net and included cells, the 'terminal nervous network'.

Deeply stained prolongations of myocardial muscle fibres enter the endocardium in many areas, and there become closely related to the nervous network. Occasional groups of ganglion cells occur.

Following unilateral vagotomy degeneration of some thick fibres and their endings was noted in both left and right atria, but no gross alteration in the network could be found. Communications between the thick fibres and the fine network are considered to occur, but are apparently slight.

I would like to thank Prof. A. Durward for his advice and criticism, and Mr R. K. Adkin for technical assistance.

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EXPLANATION OF PLATES

All figures are from whole thickness preparations of dog atrial endocardium, stained with methylene blue.

Plate 1

Fig. 1. Branching nerve fibre and end formations in left atrial endocardium.

Fig. 2. Two end formations at the end of a side branch of a thick fibre in the left atrium.

Fig. 3. End formation, right atrium. The thick fibre giving rise to the ending can be seen at the lower border of the figure; parts of the ending lie out of focus.

Fig. 4. Nervous network in the intercaval region of the right atrium.

Fig. 5. Network composed of multi-fibre strands and single fibres in the right auricular appendage.

Fig. 6. High-power photomicrograph of the nervous network in the right atrium. Note the darkly stained beads along the fibres, and an interstitial cell (*C*).

Plate 2

Fig. 7. Diffuse type of end formation ('terminal area') in the left atrium.

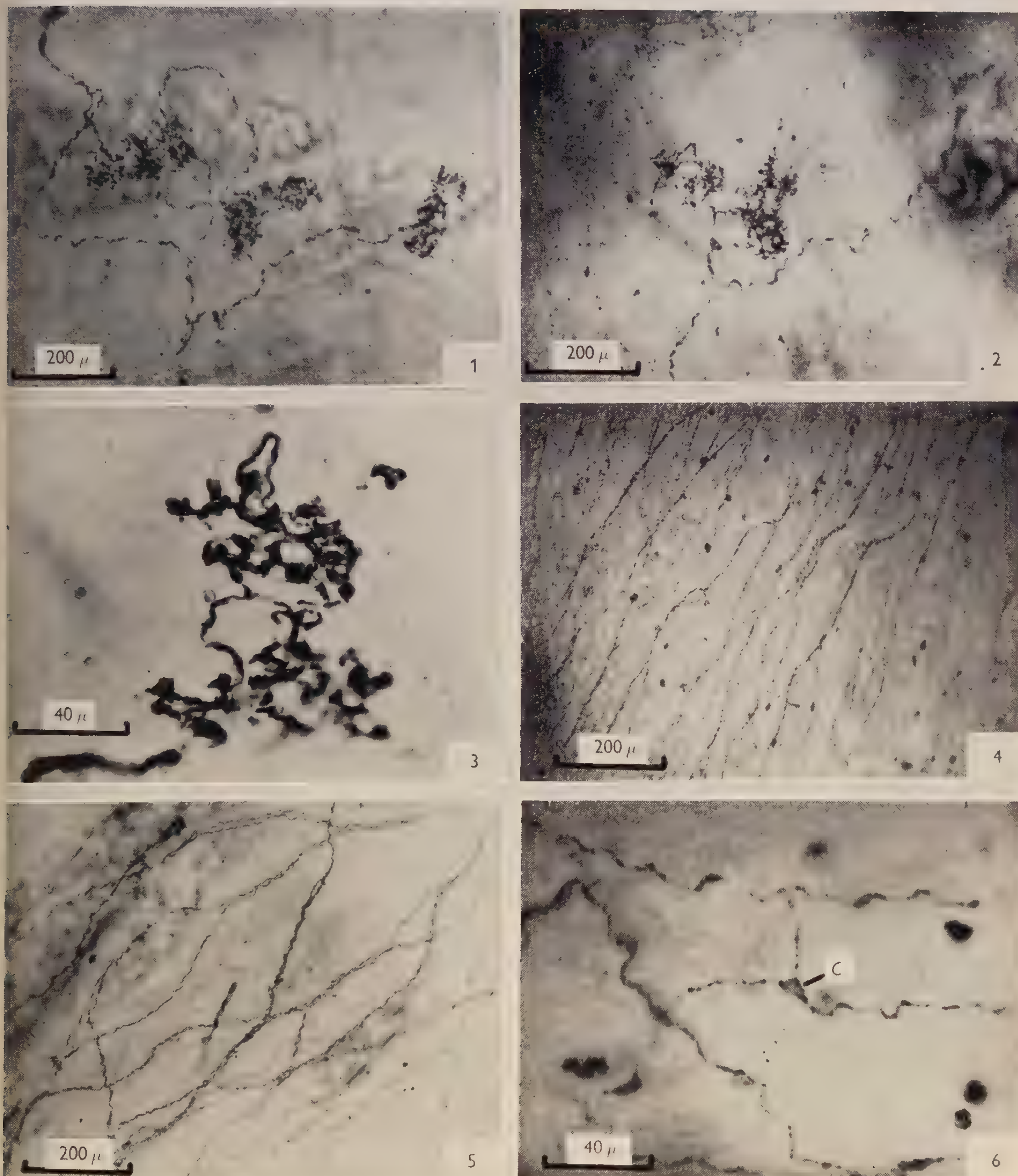
Fig. 8. Ganglion cells in the endocardium of the junctional region between the inferior vena cava and atrium; their processes pass into the nervous network.

Fig. 9. Endocardial muscle fibres in the intercaval part of the right atrium. Intercalated discs are darkly stained.

Fig. 10. Isolated endocardial muscle fibres and fine nerve fibres, right atrium.

Fig. 11. Degenerating thick nerve fibre and end formation in the left atrium of an animal which underwent a left vagotomy 9 days previously. The fibre (*F*) and ending (*E*) are disintegrating but still stain. Fibres of the terminal network (*N*) are faintly stained, but were microscopically normal. Fat deeper in the atrial wall underlies the upper part of the degenerating ending.

Fig. 12. From the same animal as fig. 11: a degenerate end formation (*E*) and its faintly outlined fibre (*F*) lie close to a small normal ending (*N*).



HOLMES—STRUCTURES IN THE ATRIAL ENDOCARDIUM OF THE DOG

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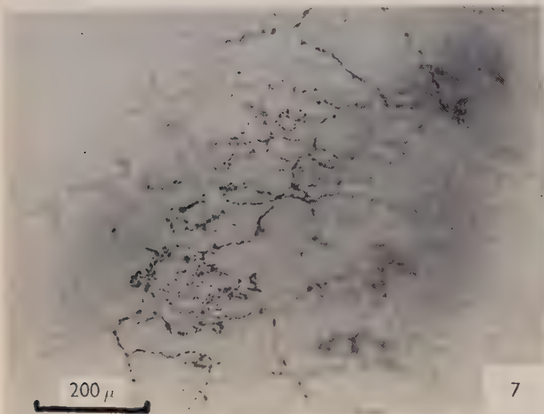

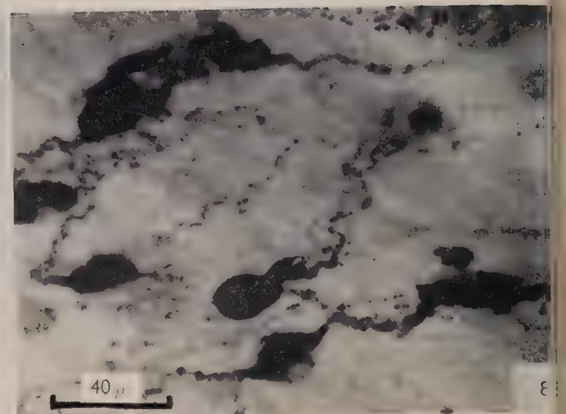

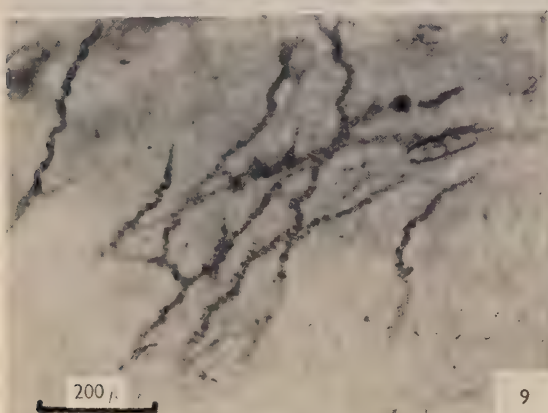

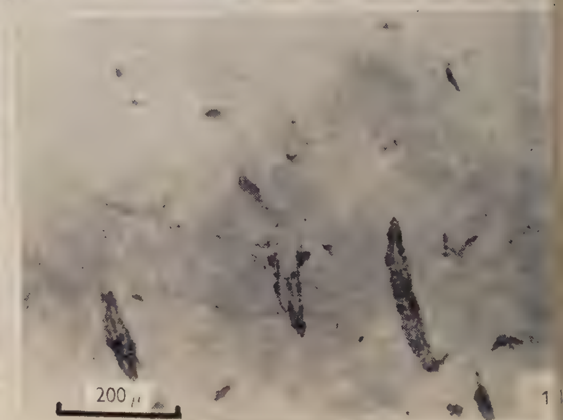

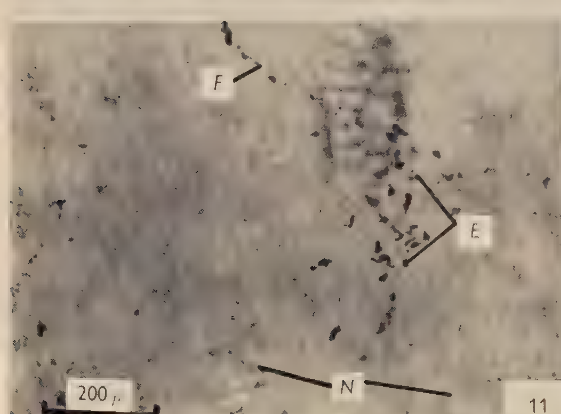

HOLMES—STRUCTURES IN THE ATRIAL ENDOCARDIUM OF THE DOG

HISTOLOGICAL EVIDENCE FOR THE INNERVATION OF HUMAN DENTINE

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The sensitivity of human dentine strongly suggests that it possesses a rich innervation. None of the workers who have attempted to demonstrate nerves in this tissue can be said to have done so unequivocally. The literature, which has been reviewed by Brashear (1937) and Bernick (1948), remains inconclusive because most workers have apparently disregarded the possibility of mistakes in their interpretation. Many histologists agree that nerves can be demonstrated in the predentine, and Bradlaw (1939) refers to intra-tubular fibrils in this region. Only a few observers who claim to have demonstrated nerves in dentine proper have supported their observations with photomicrographs. Powers (1952), who figured a retouched photomicrograph, believed that the nerves she demonstrated were situated in the dentine matrix between the tubules. Cocker & Hatten (1955), who used the same silver technique as Powers, were not prepared to say whether the silver impregnated fibres in their sections were situated in the dentinal tubules or the dentine matrix. Tojoda (1934*a*, *b*) described in some detail many types of silver impregnated fibrils within the dentinal tubules and, apparently relying on the specificity of silver for nerve tissue, seemed to regard all of them as special types of nerve endings.

It is the purpose of this paper to show that small intra-tubular nerve fibrils do extend into the dentine in close relationship to the odontoblast process (Pl. 1, fig. 8*a*, *b*), and to attempt by conventional histological techniques to eliminate the possibility of misinterpretation.

MATERIALS AND METHODS

Non-carious and slightly carious human premolars and molars from subjects between the ages 10–14 and 18–24 years were obtained immediately after extraction, which was performed under general or local anaesthesia. The approximal surfaces of some of the teeth were ground down nearly to the pulp in chilled (4° C.) normal saline. In others the roots were either cut off or large holes were drilled into the dentine in parts not required for study. The teeth were then placed in 10 % formol saline for not less than 7 days. Formic acid/sodium formate solution (Kristensen, 1948) was used for decalcification, and the majority of the teeth were double embedded in celloidin paraffin. Other embedding materials used were paraffin wax, celloidin, Nonex (Miles & Linder, 1952), or Ester Wax (Steedman, 1947). Celloidin sections were cut at a thickness of 15 μ, paraffin sections at 10 μ and Nonex and Ester Wax sections at 7 μ. The sections mounted in series were then impregnated with silver by a modified Holmes technique (Fearnhead & Linder, 1956). The quality of the

fixation was judged from the appearance of haematoxylin and eosin stained sections and the appearance of unstained sections examined by phase-contrast microscopy. The matrix of the 'translucent area' around the dentinal tubules and the cytoplasm of the odontoblast processes was demonstrated by Held's molybdic acid haematoxylin stain (Pl. 1, fig. 6). Sections of undecalcified dentine fixed in buffered pH 7·2 osmium tetroxide (Palade, 1952) and embedded in methylmethacrylate, were cut with a diamond-knife similar to that described by Fernández-Morán (1953).

Silver impregnated intra-tubular nerve fibrils were counted over measured lengths of the pulp-dentine junction in three regions of four teeth, the crown, the cervix and the root, see Appendix, Text-fig. 3, and Table 1 *a–c*.

RESULTS

Fixation

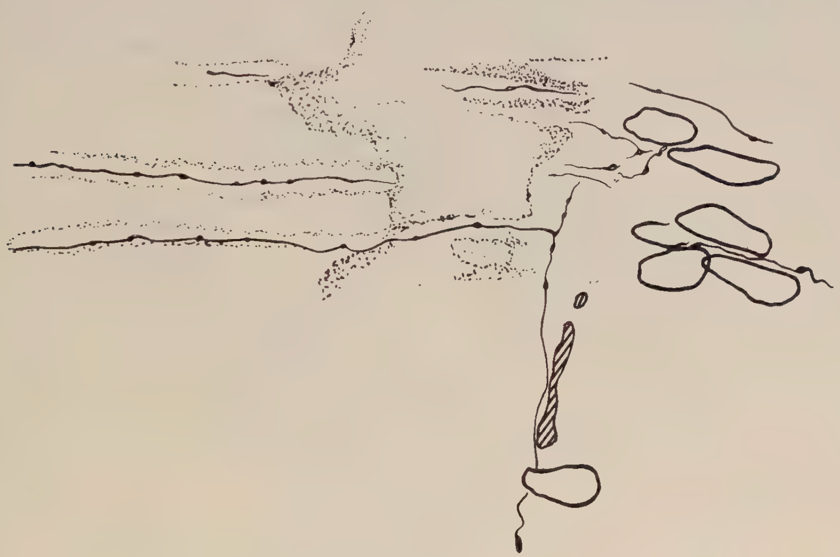
In this study a considerable amount of care was taken to select only those teeth in which fixation of the contents of the dentinal tubules was thought to be good. Unstained sections were examined with the phase-contrast microscope, and other sections were stained with haematoxylin and eosin in order to assess the quality of the fixation before impregnating the series with silver. The specimens were discarded when the pulp and odontoblasts showed vacuolation and other changes attributable to faulty fixation. In the majority of the remainder the continuous unbroken outline of the odontoblast process could be seen within many of the tubules. This was found more frequently in teeth which had been cut in half before being placed in the fixative. These specimens were regarded as being well fixed and were therefore used for the silver impregnation. In many specimens where exposure of large areas of dentine had not been obtained before fixation, the contents of the tubules appeared to be broken up into droplets, although the fixation of the pulp was often quite good. Such sections were regarded as being poorly fixed and discarded (Pl. 1, fig. 7).

Silver impregnation

In silver-treated sections of well-fixed dentine numerous small beaded fibrils could be detected lying in close relationship to the process of the odontoblast, between it and the wall of the tubule. Although in places the intra-tubular nerve fibrils were in very close association with the odontoblast process, they did not appear to be embedded in its protoplasm nor did they possess small collateral fibrils embracing it as described by Tojoda (1934*a*, *b*). Measurement of the beaded fibrils showed them to have a diameter of $0{\cdot}2\,\mu$ or less, and the beads varied in size from $0{\cdot}4$ to $0{\cdot}8\,\mu$. These fibrils therefore have the size and distinctive moniliform morphology of small terminal nerve fibrils (Weddell & Glees, 1941). All were heavily impregnated with silver and many of them could be traced in continuity with similar fibrils in the predentine which in turn could be traced to nerve fibrils situated on the surface of the predentine (Pl. 1, figs. 3, 10). The lowest beaded intra-tubular fibril illustrated in Text-fig. 1 and Pl. 1, fig. 1, was traced as far as 0·4 mm. into the dentine from the point at which it left a branch in the predentine. This is the greatest distance that any single fibril was traced when in continuity with a nerve in the pulp or predentine. Fibrils having a similar size and morphology were noted, however, much

further (1·5 mm.) in the dentine (Pl. 1, fig. 9). It was impossible to establish the continuity between these fibrils and nerves in the pulp because of the many small curvatures in the dentinal tubules and the wavy or spiral course of the beaded fibrils within the tubules (Pl. 1, fig. 8*a*, *b*).

Counts were made of intra-tubular fibrils that could be traced in continuity with nerves in the predentine or pulp. The number of nerves entering the dentinal tubules in the crown was much higher than those entering tubules in the region of the cervix. In the root, intra-tubular fibrils were only occasionally seen. The



Text-fig. 1. Camera lucida drawing of the field illustrated in Pl. 1.

statistical significance of the differences found between the numbers of fibrils in each of these three regions on ten serial sections from four teeth is dealt with in the Appendix. In all the teeth studied so far the general pattern of distribution was the same, although individual regional counts varied from tooth to tooth.

DISCUSSION

As a result of recent work it has become necessary to reconsider the classical concept of the structure of dentine. Bradford (1950, 1951, 1955) has shown that the dentinal tubule is surrounded by an area of dentine which appears translucent when ground sections are viewed by transmitted light and for which he coined the term 'translucent area'. This term is not altogether satisfactory since it refers to only one particular aspect of the physical properties of the peritubular region. Takuma, Kurahashi, Yoshioka & Yamaguchi (1956) use the term 'peritubular matrix' or 'secondary matrix' because they found that the matrix of the tubule walls appeared different in character from the rest of the dentine matrix when studied with the electron microscope. Shroff, Williamson, Bertrand & Hall (1956) prefer to use the term calcified canicular sheath to describe the walls of the dentinal tubules. Each

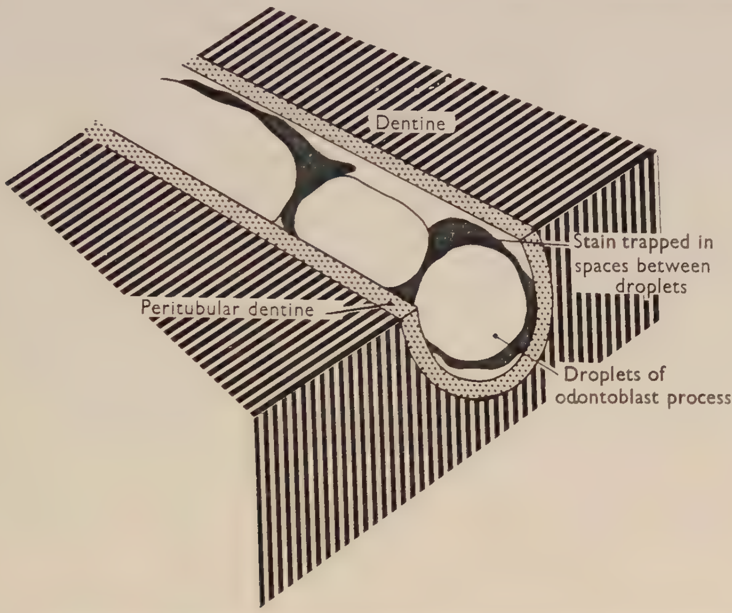
of these names is equally unsatisfactory since they refer to only one component of the structure of this region. Since little is known about the mode of formation, structure, function, or ageing processes of the dentine immediately surrounding the tubules, it might be better to employ the term 'peritubular dentine' for this region. This at least has the merit of recording where this special region may be found without placing the emphasis on any one particular feature of its structure. This term is therefore used in the remainder of this study.

The peritubular dentine consists of a delicate matrix which takes up stains more readily at its inner and outer border and is thought to be highly calcified. When dentine is decalcified for the preparation of histological sections, the delicate matrix of the peritubular dentine may be lost, disrupted, or it may remain as a sheath which can be seen to surround the odontoblast process. Sometimes the matrix appears as an empty tube when for some reason, such as poor fixation, the contents of the tubule are lost. When ultra-thin sections, cut from human dentine without previous decalcification, were examined with the electron microscope, the peritubular dentine was revealed as an electron dense region which appeared to consist of fibres orientated differently from the inter-tubular dentine matrix, (Pl. 1, fig. 4). Removal of the methacrylate, and brief (30 sec.) decalcification in sodium formate/formic acid, removed the substance responsible for the electron density and left behind what appeared to be the matrix of the peritubular dentine (Pl. 1, fig. 5). In addition, in the sections decalcified on the electron microscope grids, the junction between the peritubular dentine and the adjacent inter-tubular dentine was particularly well marked. This junction represents the position of the 'Neumanns sheath' referred to by earlier workers. These observations are obviously of considerable importance in the interpretation of the contents of dentinal tubules, since the tubules, which were originally thought to have a diameter of 3–5 μ, in reality vary in size from 1·0 to 1·5 μ at the periphery of the dentine, to 2·5–3·5 μ at the pulpal end. This of course limits the size of nerve fibrils and odontoblast processes which could be accommodated within them.

It might be argued that the silver impregnated beaded filaments were not nerve fibrils at all, but merely represented the matrix of the peritubular dentine impregnated with silver. If the matrix of the peritubular dentine were impregnated, it might be expected to envelop the odontoblast process in the form of a reticulated sheath. The fibrils shown in Pl. 1, figs. 1, 3 and 8 *a*, *b*, however, have a diameter of 0·2 μ and less, and quickly go out of focus at different levels within the same tubule. Furthermore, the matrix of the peritubular dentine can be stained by Held's molybdic acid haematoxylin method, when it can be seen to surround the odontoblast process and to have a morphology quite different from silver impregnated nerve fibrils. Some places could be found in the sections where the plane of the microtome knife had divided the dentinal tubule and its contents longitudinally. In such regions much more precise resolution of the structure of the dentinal tubules is possible. Both in sections stained with Held's molybdic acid haematoxylin and unstained sections viewed with the phase-contrast microscope, five linear structures were identified representing the inner and outer margins of the peritubular dentine, either side of the tubule, and a thicker central odontoblast process surrounded by a small space (Pl. 1, fig. 6).

In addition to these structures, in some tubules, very small spherical droplets (diameter approx. $0 \cdot 5\,\mu$) were seen closely applied to the surface of the odontoblast processes (Pl. 1, fig. 6). The spherical droplets did not stain with Held's molybdic acid stain, and appeared refractile in phase-contrast preparations. It is impossible to say whether these droplets are inclusions within the odontoblast process or whether they are lying on the surface of it. In the latter case they might represent unstained beads of an intra-tubular nerve fibril.

It was mentioned earlier that delay in fixation can cause the dentinal process of the odontoblast to break up into droplets. It was thought therefore that reduced silver in the spaces between the tubule wall and the droplets might resemble small nerve fibres (Text-fig. 2). Sections from teeth in which droplet formation was known to



Text-fig. 2. Diagram to show how silver might simulate nerve fibres by becoming reduced in the spaces between droplets formed from the odontoblast processes.

have occurred were impregnated with silver. It was hoped that silver penetrating the tubules might become trapped between droplets and tubule walls and later become reduced in this situation. This was achieved in some sections by omitting the washing and sulphite stage of the impregnation. The result is shown in Pl. 1, fig. 7. The distribution of the silver is quite distinctive in such preparations, and once seen is not likely to be confused with the appearance of silver impregnated nerve fibrils.

Many of Tojoda's illustrations show small intra-tubular beaded fibrils which have a morphology similar to the fibrils regarded as nerves in the present study. Unfortunately he did not record precisely how far into the dentine he was able to trace these fibrils. Tojoda (1934*a*, *b*), who ground away considerable amounts of enamel and dentine in order to obtain good fixation, describes and figures intra-tubular nerves having very small collateral 'sprouts' (Sprossen) clasping the odontoblast processes. This appearance can be attributed to an increase in argyro-

philia of the matrix of the peritubular dentine or of the odontoblast process, which might be due to over-heating, since it has been shown that in the region of a dental bur cutting into dentine without lubrication, temperatures as high as 368° F. can occur (Henschel, 1944). In the present study, in sections of teeth in which a small hole had been drilled prior to fixation, a zone of altered silver affinity could be clearly detected in the dentine around the hole and in the pulp cells immediately beneath it (Pl. 1, fig. 2), despite the fact that the bur had been irrigated with cold saline during cutting. The contents of the tubules in this region had a foam-like appearance almost certainly due to heating. Examined at high magnification, the contents of the tubules in this altered zone possessed a striking resemblance to the collateral sprouts figured by Tojoda. It is not meant to imply by this that grinding before fixation is valueless, but merely to indicate that care must be taken in the interpretation of the histology of dentinal tubules in close relationship to surfaces exposed in this way. Ambrose (1943) criticized the histological evidence for the innervation of dentine on the basis that the odontoblast processes themselves were being mistaken for nerve fibrils. This may well be the case in some instances. In sections prepared by the method used in the present study, however, the odontoblast process is often coloured a delicate pink; its outline is smooth and not beaded and it has a diameter of about 1 μ. The diameter of the odontoblast process is therefore approximately five times greater than the beaded nerve fibrils described in this paper. Furthermore, Pl. 1, figs. 8*a*, *b*, are photomicrographs of a section taken at different focus levels which show a small nerve fibril and a lightly coloured odontoblast process together in the same tubule. Although the silver technique used in this study gives consistent results it is impossible to say whether every nerve fibre in every section is impregnated, and this criticism is of course equally valid for any silver method. It seems certain from the results of the counts, however, that by no means every dentinal tubule contains a nerve fibril. The variation in the number of intra-tubular nerve fibrils counted in the three regions of the teeth studied has some significance in establishing the identity of these fibrils, since it is unlikely that artefacts would be distributed in such a constant pattern.

Although the junction between the enamel and dentine is especially sensitive, nerve fibrils have been demonstrated within dentinal tubules for only part of their length. The question whether the peripheral portion of the dentine is innervated must therefore be regarded as still unsettled. Fernández-Morán (1952) and others have shown that submicroscopic nerve fibrils exist in the central nervous system, and De Robertis & Sotelo (1952) have shown that the pseudopodal processes of neurites grown in tissue culture may possess submicroscopic extensions of their cytoplasm. It is quite possible, therefore, that terminal nerve fibrils too small to be resolved by optical microscopy may extend as far as the enamel-dentine or cementum-dentine junction as continuations of the intra-tubular nerve fibrils described in this work. It was hoped that silver deposited on the surfaces of such fibrils might bring them within the range of the optical microscope since, according to Romanes (1950), silver deposited on nerve fibrils during physical development results in an increase in their diameter. It is of interest in this respect that in many of the silver preparations some of the dentinal tubules contained extremely thin fibrils very near the dentine-enamel junction (Pl. 1, fig. 9). These fibres could not be identified as nerve fibres

with the light microscope, but it is possible that the greater resolving power of the electron microscope may help to elucidate their true character. It is also worthy of note that occasionally dentinal tubules containing more than one fibril have been discovered by electron microscopical methods (Scott, 1955). Consideration of this problem would be incomplete without mention of the recent observations of Shroff *et al.* (1956). These workers examined shadowed replicas of freshly fractured dentine, polished surfaces of dentine and ultra-thin sections of decalcified dentine with the electron microscope. In their preparations they were able to identify several distinct structural layers in the odontoblast process. They describe these layers as a central core of a labile protein nature surrounded by a thin organic sheath which in turn is surrounded by a thick material possessing some of the properties of myelin. Around this 'myelin-like' layer is a thin outer sheath composed of fibrils which appear to be collagen. The myelin-like sheath is osmiophilic, soluble in hot alcohol, and stains blue with acidified methylene-blue, the latter apparently being regarded as a specific stain for myelin. On the basis of these observations they conclude that there is a close similarity between this layered submicroscopic structure of the odontoblast and the submicroscopic structure of vertebrate nerve fibrils as described by Fernández Morán (1950*a*, *b*). Shroff *et al.* (1956) suggest therefore that if their observations are correct the odontoblasts may function as some form of receptor cell, a suggestion which revives the hypothesis put forward by Hopewell-Smith (1893) and supported more recently by Philipp (1955). The evidence used by Shroff and his co-workers is open to criticism, however; for example, it is very doubtful whether osmiophilia, solubility in hot alcohol, or staining with acidified methylene blue can be regarded as properties possessed by myelin alone. It is also difficult to see how the suggestion put forward by these workers can be reconciled with the observations of Geren (1954) who has demonstrated the formation of myelin sheaths from Schwann cell membranes.

Finally, the replica method does not always permit an accurate interpretation of soft tissue structure. It is important, therefore, to develop techniques for preparing ultra-thin sections of dentine suitable for electron microscopy in which the contents of the tubules are undisturbed. Fixation of the dentine is obviously of extreme importance to this aspect of the study and unfortunately the zone of dentine nearest the enamel is most inaccessible to fixatives. Consequently, until the difficulties involved in obtaining rapid fixation in the peripheral region of the dentine are overcome, it is impossible to draw very precise conclusions about its histology.

SUMMARY

Using a modified Holmes silver technique very small beaded intra-tubular nerve fibrils have been demonstrated in human dentine. These fibrils have an approximate diameter of $0{\cdot}2\,\mu$ and are situated in the tubules between the odontoblast process and tubule wall. They were traced in continuity from the predentine into the dentinal tubules for varying distances up to 0·4 mm. Fibres with similar morphology were identified even farther into the dentine. For the present it is not possible to determine whether fine terminal filaments from the intra-tubular nerve fibrils extend as far as the enamel-dentine or cementum-dentine junction. Using various conventional

histological methods care was taken to exclude sources of artefact which could lead to misinterpretation of the silver impregnated fibrils.

I wish to express my thanks to Prof. A. E. W. Miles and Prof. R. J. Harrison for their encouragement, helpful advice and criticism. I am indebted to Dr P. Grodzinski and Dr J. F. H. Custers of Industrial Distributors Ltd. for the preparation of the diamond knife with which the sections of undecalcified dentine were cut. My thanks are also due to Mr W. K. Mansfield of Queen Mary College for his generous assistance in the use of the electron microscope. Text-fig. 2 was drawn by Miss P. Archer, and the expenses were partly defrayed by a grant from the Yarrow Research Fund, The London Hospital Medical College.

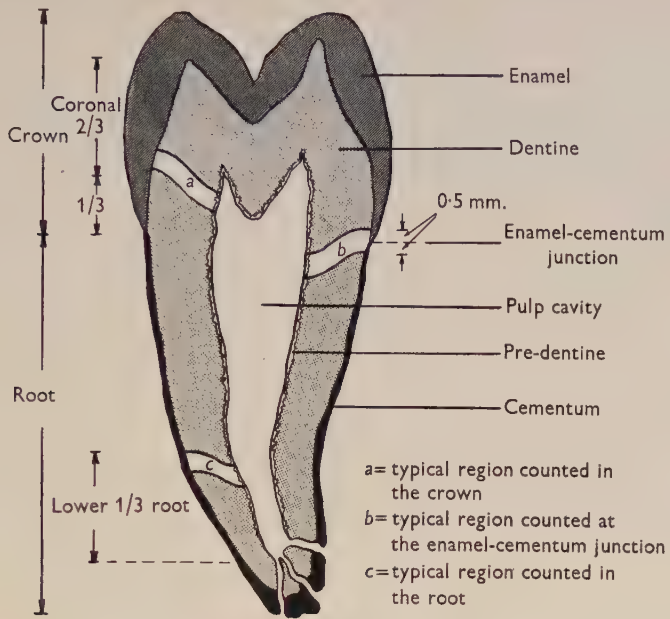
APPENDIX

Dentinal tubule and intra-tubular nerve fibril counts

Ten longitudinal sections, 10 μ thick, were selected from the mid-portion of each of the four teeth on which the counts were made. Ten different fields, measuring 0·1 mm. in diameter, were chosen in each of the three regions, five fields being selected from opposite sides of the tooth for each region. The method of selecting fields was determined to a large extent by the plane of the section because the dentinal tubules do not pursue a straight course. Fields were chosen therefore from areas where the dentinal tubules were cut longitudinally at the junction between the predentine and dentine. The counts of intra-tubular nerve fibrils and dentinal tubules are listed in Table 1 *a*–*c*. These totals represent the result of 100 fields within each region for each tooth counted.

In order to avoid overlapping of regions the third of the crown nearest the root was never included in the crown counts (Text-fig. 3). The region designated enamel-cementum junction included dentinal tubules which were situated within 0·5 mm. from the true junction in both directions, that is, coronally or rootwards. In practice these tubules were located by first moving the junction on the outer surface of the tooth into the centre of the field, and then following the curvature of the dentinal tubules by manipulating the stage micrometer until the predentine-dentine junction was reached. In the root the counts were taken from the third of the root farthest away from the crown. Differences in the totals between the crown region and the enamel-cementum junction (Table 1 *a*) and the enamel-cementum junction and the root region (Table 1 *b*) were tested separately by means of the χ^2 test. It will be seen that only in one case (Table 1 *b*), specimen 2, where the χ^2 value is 0·751, is the P value greater than 0·3. In all other cases the statistical value can be regarded as highly significant. For completeness the χ^2 value for the difference between crown and root of specimen 2 is included (Table 1 *c*). Calculated with 1 D.F. the value for $P = 0{\cdot}05$ is 3·84.

I am greatly indebted to Miss V. Cane, Department of Mathematics, Queen Mary College, London, and to Dr F. Steel, Anatomy Department, London Hospital Medical College, for their considerable help and advice with the statistical treatment.



Text-fig. 3.

Table 1*a*

	Crown		Enamel-cementum junction		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
1	212	2563	28	2722	152·49
2	105	2197	5	2460	102·89
3	90	2220	15	2348	57·91
4	188	2301	24	2299	71·539

Table 1*b*

	Enamel-cementum junction		Root		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
1	28	2722	5	2379	13·14
2	5	2460	2	1998	0·751
3	15	2348	2	2201	8·887
4	24	2299	2	8679	16·72

Table 1*c*

	Crown		Root		
Specimen	Intra-tubular nerve fibrils	Tubules	Intra-tubular nerve fibrils	Tubules	χ^2
2	105	2197	2	1998	89·81

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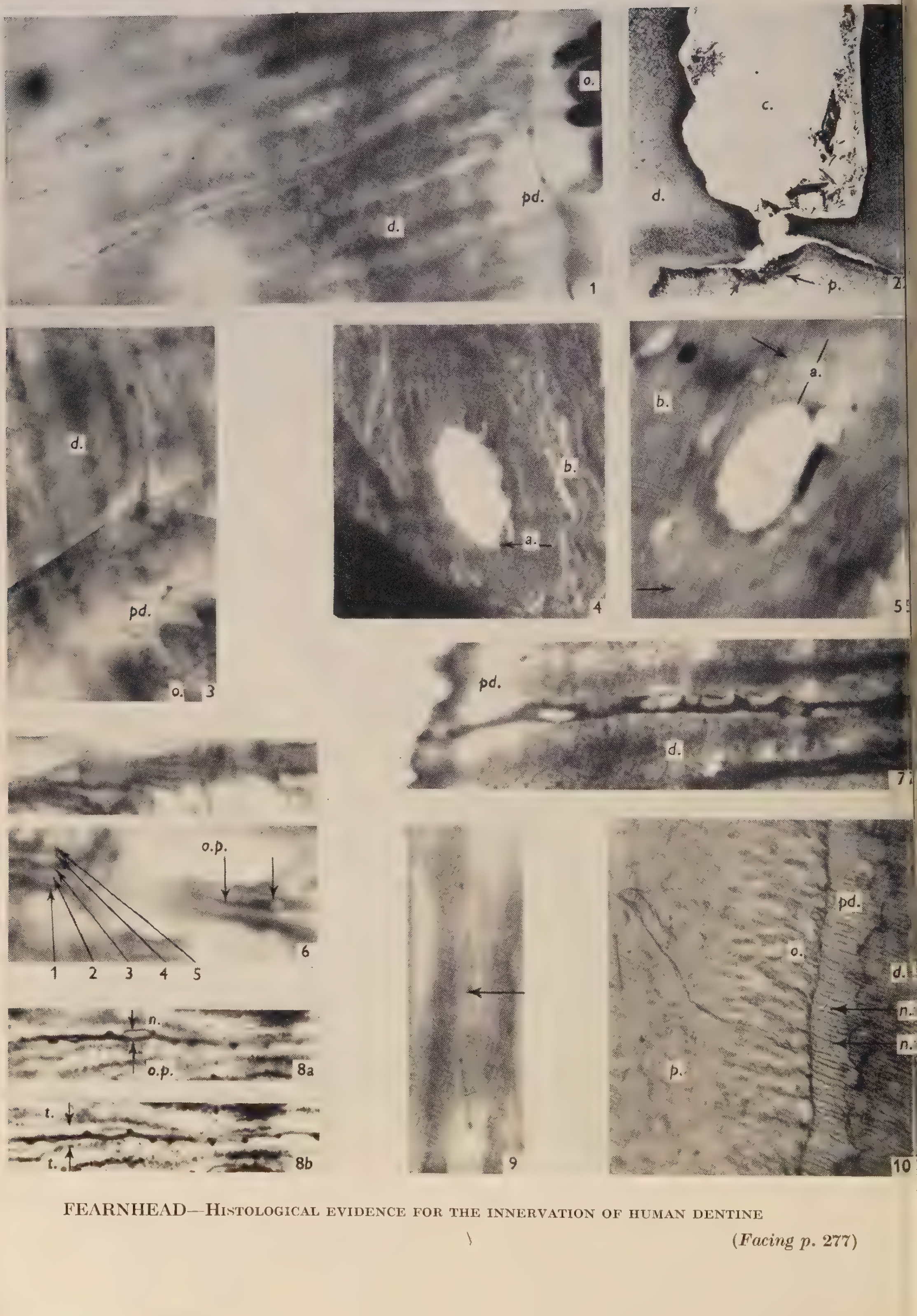

FEARNHEAD—Histological evidence for the innervation of human dentine

(*Facing p.* 277)

LIST OF ABBREVIATIONS

a. 'translucent area' or peritubular dentine
b. inter-tubular dentine matrix
c. cavity
d. dentine
o. odontoblasts
pd. predentine
p. pulp
op. odontoblast process
n. nerve fibril
t. dentinal tubule

EXPLANATION OF PLATE

Fig. 1. See also Text-fig. 1. Two photomicrographs taken at different focus levels and joined to show intra-tubular beaded fibrils, and similar fibrils in predentine. Silver impregnated section from decalcified human premolar. (×1500.)

Fig. 2. Section of dentine and pulp from a tooth in which a small hole was drilled prior to fixation. Note the difference in the character of the staining of the dentine adjacent to the hole, and the zone of damage in the pulp indicated by the small arrows. Silver impregnation. (×20.)

Fig. 3. Two photomicrographs of the same field taken at different focus levels and joined to show a beaded intra-tubular nerve fibril arising as a branch from a similar fibril in the predentine. Silver impregnation human premolar. (×1500.)

Fig. 4. Electron micrograph of undecalcified dentine from the crown of a lower third molar tooth from a male aged 21 years. The area (*a*) around the tubule which appears electron dense represents the peritubular dentine equivalent to the 'translucent area' seen in ground sections. The arrow from (*a*) ends on the tubule wall. (×10,000.)

Fig. 5. Electron micrograph of a section adjacent to that in fig. 4. The methacrylate was removed and the section decalcified for 30 sec. in 10% sodium formate/formic acid solution. Note the electron dense character of area (*a*) in fig. 4 has disappeared and the junction between the dentine matrix and the region of the 'peritubular dentine' is more clearly defined. Arrows indicate the position of 'Neumann's sheath'. (×10,000.)

Fig. 6. Decalcified section of dentine from human premolar stained with Held's molybdic acid haematoxylin. The five lines represent the junctions between the outer margin of inter-tubular dentine and the peritubular dentine, the matrix of the peritubular dentine, and the tubule wall, and the odontoblast process. The arrow indicates a small, spherical, unstained droplet closely applied to the surface of the odontoblast process. (×1800.)

Fig. 7. Reduced silver trapped in the dentinal tubule between globular remnants of the odontoblast process. Decalcified section of human premolar, silver impregnation. (×1500.) See also Text-fig. 2.

Fig. 8*a*, *b*. Two photomicrographs of the same field at different focus levels. A silver impregnated beaded fibril which has the characteristic morphology of a small nerve, and a more delicately impregnated odontoblast process in the same tubule. This appearance is not due to trapped stain, or the matrix of the 'peritubular dentine', nor is it due to staining of the odontoblast process since this can still be identified as a discrete object within the same tubule. Decalcified section of dentine from a human premolar approximately 0·4 mm. from the predentine. (×1000.)

Fig. 9. A small impregnated beaded fibril with a morphology similar to a very small nerve fibre in a dentinal tubule. This fibre was found situated 1·5 mm. from the pulp-dentinal junction. (×1800.)

Fig. 10. A nerve fibre in the pulp; branches from this nerve are situated between the odontoblasts and on the surface of the predentine, and small terminal branches with short lengths of their course in focus can be seen lying across the dentinal tubules in the predentine. Silver impregnation, human molar. (×400.)

THE EFFECT OF LARGE DOSES OF CORTISONE ON THE NEONATAL RAT

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The observations here described were made in the course of experiments primarily undertaken to test the effect of large doses of cortisone on microglial immigration and transformation in the neonatal rat brain. Whilst the expected suppression of Hortega's 'fountains' was found to occur (Field, 1954), marked and rapid growth developmental changes were outstanding.

MATERIAL AND METHODS

Altogether more than seventy litters of rats have been used. In each case half the animals have been injected with cortisone acetate suspension (Merck or Roussel) and the remainder with the corresponding suspending medium. Injections were made subcutaneously in the mid-dorsal region at some time in the first week and usually within the first 3 days of birth, when the animals were some 5–7 g. in weight. Doses of 0·01–0·1 ml. (0·25–2·5 mg.) were used, and such an inoculum could be seen to remain as a deposit around the site of injection for 3–5 days (Pl. 1, fig. 1). Animals were weighed daily in batches. Experimental and control animals were sacrificed at intervals for examination of pituitary, pineal, thyroid, liver, mesenteric lymph node, thymus, spleen, adrenal, upper end of tibia, brain and teeth. Bouin and Zenker fixed material has been stained by haematoxylin and eosin, Wohlbach's Giemsa and the Schiff periodic acid method.

Pituitaries from earlier experiments were fixed in Bouin or Zenker's fluid, but it was later found that more precise differentiation between types of cell was obtainable after formol-sublimate fixation. It is preferable not to pass the pituitaries through iodine but to remove mercury deposit from the sections on the slide. Treatment of the whole pituitary with Lugol's solution results in an artefact at the periphery of the sections when they come to be stained. All pituitaries were cut (3μ) horizontally, i.e. parallel to the base of the skull, and in making differential counts care was taken that equivalent fields were examined under similar conditions. In every pituitary more than 2500 cells were counted. Krichesky's modification of Mallory's aniline blue collagen stain (1931) and Heidenhain's azan method both gave good results after formol-sublimate fixation. Considerable difficulty was, however, experienced in preparing good, well and uniformly stained sections of the smallest pituitaries at $3–4\mu$.

RESULTS

Within 24 hr. of injection of 0·01 ml. (0·25 mg.) of cortisone to 5–6 g. animals a diminished weight increase was apparent. With doses of 0·1 ml. there was a consistent weight loss and the treated animals had a lax skin which wrinkled readily on pinching

it up. These changes gradually became more pronounced, and in general were more striking the younger the animals at the time of injection (Pl. 1, fig. 1; Text-fig. 1). Exophthalmos was noted 2 or 3 days after injection.

When all the cortisone at the injection site had disappeared the animals began to put on weight more rapidly (unless the initial dose had been an overwhelming one). In many groups, therefore, second or third doses were given, never greater than 0·1 ml. (2·5 mg.) and spaced out at appropriate intervals so that the weights of the experimental animals remained well below those of their litter-mate controls. In this way a loss of 50–60 % of presumptive weight could be induced. Such animals with severe retardation of growth often died spontaneously and without apparent cause. Only on two occasions was a localized skin abscess formed at the site of inoculation. The outstanding features of the retarded animals were: (1) failure of hair growth; (2) laxness and thinness of the skin, producing a wizened and shrivelled appearance; (3) abnormal tooth growth; (4) exophthalmos. No differences were found between the sexes in the response to cortisone.

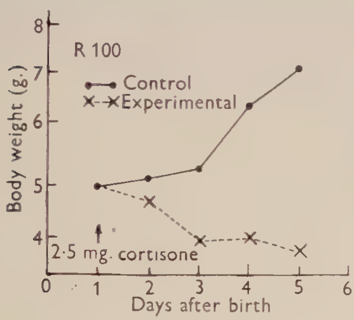

Fig. 1

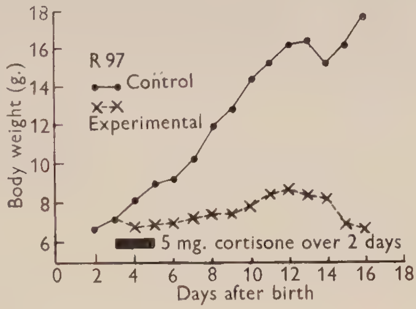

Fig. 2

Text-fig. 1. R 100. 2·5 mg. cortisone given on the day of birth. Note the immediate effect on gain in body weight. Animals sacrificed at 5th day.

Text-fig. 2. R 97. 5 mg. cortisone given from 3rd to 5th days after birth. The incisura on the growth curve of the control animals at 13 days was due to inadvertent restriction of the mother's food intake for 24 hr. This seems to have led to a fatal issues for the experimental animals at 16 days.

Failure of hair growth

A large initial dose (2·5 mg.) often resulted in a generalized failure of hair growth so that the animal had a pink and naked appearance as compared with controls. This state of affairs persisted for about a month when a focus of luxuriant hair growth would often appear at the injection site (or sites) and spread rapidly in a few days (Pl. 1, fig. 2). Histologically the skin was found to be much thinned in all its layers, especially in the stratum germinativum and stratum granulosum, and subcutaneous fat was much diminished. The hair follicles were much thinner and less cellular, whilst mitotic figures were conspicuously absent in the treated animals (cf. Green & Ghadially, 1951). On the other hand, trypan stuffed macrophages were not altered, either in number or degree of filling as compared with controls. The failure of hair and skin growth seems attributable to the virtual extinction of mitotic activity whilst the later luxuriant growth may be a 'rebound' phenomenon.

Abnormal tooth growth

A full investigation of the gross and microscopic tooth and jaw changes is in progress in collaboration with Prof. A. I. Darling, and only the most conspicuous features are given here. Treated animals showed a greater apparent eruption of both upper and lower incisors within a few days (Pl. 1, fig. 3*a*, *b*). But only in those animals whose weights had been reduced to less than 40–50 % of normal was well-marked deformity produced in about a month (Pl. 1, fig. 4). The mechanism whereby this deformity is produced is not certain, but it seems likely that in part, at least, it results from a failure of attrition owing to inaccurate opposition of the incisal margins (A. I. Darling). Sometimes the tusk-like incisors became long enough to perforate the palate. The molar teeth appeared normal.

Exophthalmos

Bulging of the eyes was noted within a day or two of injection long before the lids had opened. It became more marked as animals fell behind in their growth, and in many cases led to opening of the lids 2 or 3 days before this took place in the control litter-mates, a particularly striking circumstance in view of the general backwardness of the experimental animals. Histologically the orbits appeared normal.

Pituitary gland

The pituitaries of experimental animals were smaller than those of their littermate controls. Acidophiles were obviously decreased in number, size and intensity of staining in the experimental animals. Whilst acidophiles have always been easy to stain, the differentiation of basophiles from chromophobes has never been as clear-cut in animals under about 50 days of age as in adults. A further difficulty has been the occurrence of many seemingly basophilic cells with quite distinct eosinophilic granules within their cytoplasm. These are especially well brought out by the method of Krichesky (1931) and less distinctly by azan staining. They have been reported by many workers and are fully considered and depicted by Romeis (1940, p. 127). Whilst Baker & Everett (1944) see the existence of such cells as 'disastrous to the currently held concept that basophiles and acidophiles represent independent cell lines' (p. 263), there can be no doubt of their reality and of the further difficulty it adds to the making of reliable pituitary cell counts.

Even more serious are the variations in the picture which may be induced by minor differences in technique. A good deal depends on the degree of differentiation to which the slide is submitted and individual variation cannot be altogether eliminated. It is interesting that the well-known colour illustration given by Maurer & Lewis (1922) and reproduced in Maximow & Bloom (1936, p. 298) depicts red blood cells stained intensely blue. Lillie (1948) has also drawn attention to the differences in results obtained by different methods, and it is a striking fact that acidophiles are well demonstrated by Heidenhain's iron haematoxylin which stains their granules deeply. In view of these difficulties and the additional one of precise orientation of small glands for purposes of sectioning and counting, it is not surprising that substantial variations in the proportion of basophile cells in the pituitary of the young rat occur in the literature, e.g. 8·6 % (Wolfe, 1935), $12{\cdot}3 \pm 2{\cdot}9$ %

(Baker & Everett, 1944), 4 % (Sharpless & Hopson 1940). Rodriquez (1937), indeed, was unable to demonstrate any granular basophilic cells in the pituitary of the rat or guinea-pig, but only a few diffusely stained basophiles. He also drew attention to the marked species differences shown by the gland and in particular to the variation in proportion of basophiles. The author too has not been able to demonstrate distinctly granular basophile cells in the pituitaries of young rats (up to about 40 days). More recently Petersen & Weiss (1955) have investigated the stains of anterior pituitary cells with carefully buffered solutions. They have shown that while stains may be specific for one cell type at a specially arranged pH, considerable overlap does occur and that 'both types of granules are amphoteric, staining with either acid or basic dye' (p. 108).

Some of the 'basophiles' in formol-sublimate fixed pituitaries were pale and on the whole rather large rounded looking cells. As opposed to these were numbers of small angular and deeply stained cells often giving the impression of being fitted in between the larger cells. In making differential counts cells were therefore enumerated under the headings of eosinophiles, basophiles and hyperbasophiles. Eosinophiles were always well and consistently stained. Results are set out in Table 1.

It will be seen that there was a consistent diminution in eosinophile cells of high statistical significance but that no significant alteration in the relative numbers of basophiles or hyperbasophiles could be established. In addition to being reduced in number, the eosinophiles were smaller and often less well populated with characteristic granules than those of the normal controls. Basophiles, on the other hand, appeared morphologically unchanged.

Lymphatic system

Profound changes were noted in the lymphatic system but their consideration lies outside the scope of this paper.

DISCUSSION

The present observations confirm and extend those reported Palmer, Katonah & Angrist (1951) on the effects of cortisone on infant rats, and are closely similar to those of Karnofsky, Ridgway & Patterson (1951) on the effect of the drug on chick embryos. With regard to the rat the present work draws attention to (*a*) changes in hair growth, (*b*) exophthalmos and its importance as a factor in determining precocious eye opening, (*c*) development of tusk-like incisors, (*d*) marked diminution in eosinophile cells of the pituitary gland.

In the chick, Karnofsky *et al.* found that eggs which had been injected showed inhibition of embryo growth with markedly deficient feather formation, and the small shrivelled embryo did not survive hatching. The eyes, brain and heart were disproportionately large. Though the embryos were not susceptible to an inhibitory action of cortisone until the 8th–10th day, a time at which endocrine activity appears to come on, the authors regarded it as unlikely that cortisone acted primarily by modifying or inhibiting the function of endocrine glands. The appearance of both inhibited chicks and neonatal rats certainly suggests suppression of pituitary function, though a possible alternative explanation might be a generalized depression of mitotic activity throughout the body perhaps secondary to enhanced protein catabolism (see below).

It is known that growth of very young rats is not completely under pituitary control, for rats which are hypophysectomized at 18 days continue to grow for about a week postoperatively, whereas those over one month at the time of operation cease growing immediately (Collip, Selye & Thompson, 1933). Van Eck (1940) removed the pituitary from the 9-day-old rat and found that such animals doubled their body weight during the first 3 postoperative weeks. Walker, Simpson, Willet & Evans, (1950) succeeded in performing the operation at 6 days and found that such animals

Table 1

Controls

No.	Sex	Age when examined (days)	Eosinophiles	Basophiles	Hyperbasophiles	Total basophiles	Chromophobes
292 B	M.	45	35·320	6·360	3·580	9·940	54·740
292 G	M.	52	37·115	5·635	4·435	10·070	52·815
292 F	F	52	29·782	4·295	3·352	7·647	62·571
277 D	F.	30	40·910	6·335	3·247	9·582	49·508
277 F	M.	30	35·450	5·680	0·960	6·640	57·910
276 B	F.	30	37·980	5·830	1·890	7·720	54·340
276 F	F.	30	41·380	7·030	2·513	9·543	49·077
276 D	M.	30	37·125	6·575	3·365	9·940	52·935
268 J	M.	36	34·375	5·660	1·959	7·619	58·006
268 K	F.	36	37·170	6·290	3·182	9·472	53·358
284 E	M.	29	44·092	5·425	2·444	7·869	48·039
284 F	F.	29	33·766	4·150	1·630	5·880	60·354
293 E	F.	24	34·335	5·100	1·986	7·086	55·579
293 G	F.	24	25·195	4·920	1·718	6·638	68·167
304 D	F.	35	34·516	5·140	2·330	7·470	55·014
303 F	M.	30	31·973	5·764	3·965	9·729	58·298
300 D	F.	13	30·985	7·149	·1000	7·249	61·766

Cortisone animals

No.	Sex	Age when exmined (days)	Total cortisone (mg.)	Eosinophiles	Basophiles	Hyperbasophile	Total basophiles	Chromophobes
292 A	F.	45	17·5 in 35 days	23·74	5·250	5·325	10·575	65·685
292 C	M.	52		20·76	5·3	5·420	10·720	68·520
292 D	M.	52		24·32	4·451	5·76	10·211	65·469
292 E	F.	52		19·136	2·716	3·366	6·083	74·782
277 G	M.	30	3·75 in 19 days	25·135	4·135	2·346	6·481	68·384
277 C	F.	30		25·28	5·695	2·89	8·585	66·135
277 E	F.	30		22·258	4·59	1·811	6·401	71·341
276 A	F.	30	4·5 in 19 days	19·735	5·74	1·55	7·29	72·975
276 H	M.	30		20·488	6·98	1·538	8·518	70·994
276 I	M.	30		21·180	5·78	1·351	7·131	71·689
276 G	F.	30		20·917	7·845	1·467	9·312	69·771
268 G	M.	36	6·75 in 29 days	20·1	3·575	2·193	5·768	74·132
268 E	F.	36		17·915	5·54	1·547	7·087	74·998
268 H	M.	36		24·098	6·93	3·255	10·185	
284 C	M.	29	5 in 14 days	22·158	—	—	—	—
284 B	M.	29		25·006	—	—	—	—
284 D	F.	29		18·07	4·905	2·302	7·207	74·723
293 A	M.	24	3·75 in 5 days	15·12	2·18	1·516	3·696	81·184
293 D	F.	24		16·717	5·02	2·0237	7·057	76·226
293 C	F.	24		12·415	2·288	1·111	3·399	84·186
304 C	M.	35	25 in 31 days	15·169	4·530	3·469	7·999	76·832
303 E	M.	30	1·5 in 5 days	19·642	7·460	2·190	9·650	70·708
300 C	F.	13	2·5 in 4 days	15·881	6·390	2·300	8·690	75·429

Application of Fisher's t test shows that there is a highly significant reduction in eosinophiles ($P < 0{\cdot}001$).

continued to grow at 50% of the normal rate until 30 days. Thereafter, gain in weight ceased, whereas animals hypophysectomized on the 28th day failed at once to gain weight. In the 6-day animals they also found a disturbed cranioneural relationship, in that the skull appeared more globular and lagged in development behind the enclosed brain which seemed in consequence subjected to compression.

The present cortisone-treated animals, on the other hand, lost weight immediately and substantially if the dosage was heavy (Text-fig. 1), and this must mean that pituitary suppression cannot be the only factor involved. It is now recognized that cortisone has a catabolic or anti-anabolic effect upon protein metabolism (Long, Katzin & Fry, 1940; Hoberman, 1950; Clark, 1950; Silber & Porter, 1953) and this seems likely to be the immediate cause of failure to grow. The later continued failure may be consequent upon pituitary hypofunction, for histogically the pituitary cells are smaller than normal and poorly developed, whilst mitotic figures are virtually absent. Acidophiles are diminished in number and many of them appear partially degranulated or their granules stain an orange colour rather than deep red by the azan or Krichesky methods.

The cytological changes in the pituitary here reported differ in important respects from those found by other workers. Thus Golden & Bondy (1952) gave adult rats 5 mg. of cortisone per day for 10 days and found a significant increase in the percentage of basophile cells. Exposure to cold induced a similar change, but ACTH failed to do so. The acidophiles were uninfluenced by these treatments. On the other hand, Koneff (1944) working with 26-day-old male rats found that administration of ACTH over 30 days resulted in a significant diminution in the size of basophile cells which also seemed to be decreased in number. Again other cell types were unaffected. Since the treated animals showed stunted growth, much as if they had been starved, Koneff examined the pituitaries of rats kept for 30 days on a restricted diet but found no changes comparable with those produced by ACTH.

More recently, Kallman & Gordon (1954) have studied the morphological effects produced by the implantation of cortisone acetate pellets near the pituitary in the adult rat. They found the basophiles to undergo striking and extensive changes characterized by increased numbers and the development of large hyaline vacuoles closely resembling those found in castrated animals, the so-called 'signet ring cells'. The early changes apparent in acidophiles were quite variable and of a qualitative nature.

The difference between these results and those in the present work underlines yet again the great importance of design of experiment in evaluating hormonal effects.

Walker *et al.* (1950) did not observe the formation of tusk-like teeth in their 6-day hypophysectomized rats and no explanation can be offered for this. On the other hand, one or two severely inhibited animals have shown a 'round-headed' or 'moonface' appearance apparently similar to the more pronounced alteration produced in the 6-day hypophysectomized animals of Walker *et al.*

The exophthalmos produced in 28-day-old rats by repeated cortisone injections has been shown by Boas & Scow (1954) to be due to a retarded growth of the skull and orbit as compared with normal increase in size of the eyeball, so that it is in effect an apparent rather than a real exophthalmos. In the present series of animals exophthalmos came on within 2 or 3 days of injection, and this suggests an additional

and more rapid mechanism. Aterman & Greenberg (1953) are inclined to attribute cortisone-induced exophthalmos to a direct alteration of the physico-chemical condition of the tissues within the orbit. Thyrotrophin is known to induce exophthalmos (Heinemann, 1937). Moreover, cortisone depresses thyroid activity ('corticogenic hypothyroidism') and it might be supposed that a compensatory oversecretion of thyrotrophic hormone takes place leading to exophthalmos. Brown-Grant, Harris & Reichlin (1954), however, are of the opinion that cortisone and ACTH act mainly by suppressing thyrotrophic hormone secretion and it seems unlikely that the inactive looking pituitary of the dwarfed exophthalmic rat produces an excess of the hormone. The thyroid gland of markedly retarded rats does not show any pronounced abnormality. The immediate cause of the exophthalmos cannot therefore be settled.

SUMMARY AND CONCLUSIONS

1. Large doses of cortisone produce a marked and immediate retardation of growth in the neonatal rat.
2. Exophthalmos, failure of hair growth, poorly developed subcutaneous fat and thin shrivelled skin are features of the retarded animals.
3. When weight is less than about 50% of litter-mate controls severe incisor tooth deformities appear; minor apparent precocity of eruption is seen within 2 or 3 days of the initial cortisone injection.
4. Administration of cortisone leads to a marked decrease in the number of eosinophile cells (which also show a diminished granular content) but no significant change in basophiles.
5. The possible role of the pituitary depression in producing the syndrome is discussed and comparison made with the effects of hypophysectomy in the young rat. It is suggested that cortisone acts only in part by producing a functional hypophysectomy.

The author is indebted to Prof. J. M. Yoffey for histological facilities in the department, and to Mrs M. C. Bruce for much assistance with the histological investigations. The Medical Research Council has kindly supplied the cortisone used in this work and the author would like to thank them for this and for defraying the cost of the investigation.

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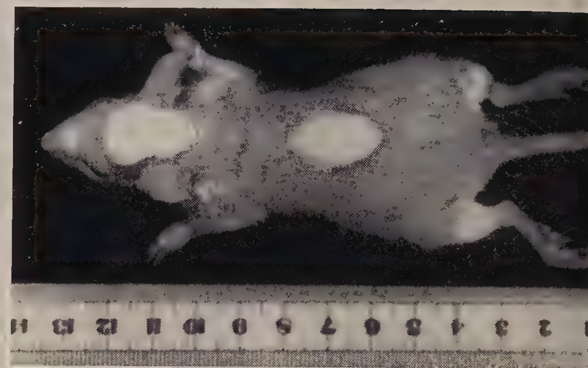

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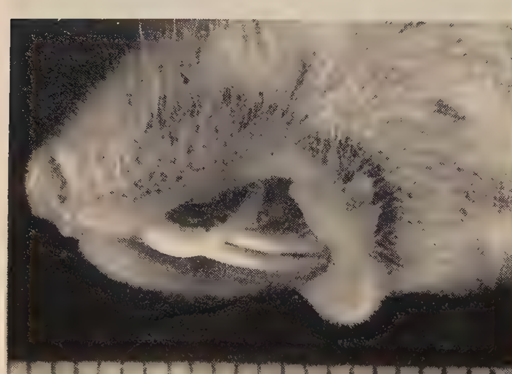

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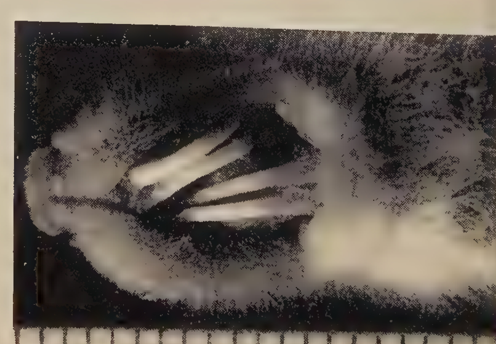

FIELD—THE EFFECT OF LARGE DOSES OF CORTISONE ON THE NEONATAL RAT

(*Facing p.* 285)

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EXPLANATION OF PLATE

Fig. 1. R 156. Litter-mates 9 days old. The smaller animal has been injected with 0·1 ml. cortisone (2·5 mg.) on the day of birth. Islands of subcutaneous cortisone are still visible. Note the prominence of the eyes of the experimental animal as compared with the control.

Fig. 2. R 113. 30 days old. Injected with 0·08 ml. (2·0 mg.) of cortisone subcutaneously into the back during the first 9 days of post-natal life. Patches of mature hair have appeared at the injection sites.

Fig. 3. R 150. 14-day-old litter-mates. (*a*) Control weighing 25·1 g. (*b*) Experimental animal injected on the 4th and 11th days with 0·1 ml. (2·5 mg.) of cortisone subcutaneously into back. Weight 13·1 g. Note prominence of incisors both upper and lower. The upper show commencing irregularity. This became more marked later.

Fig. 4. R 112. 60 days old. Weight 55 g. as compared with 211 g. litter-mates. Note the tusk-like incisors seen in lateral view (*a*) and from the ventral aspect (*b*). The upper incisors impinge upon the palate and have ulcerated into it.

ELECTROMYOGRAPHY OF CERTAIN HIP MUSCLES

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INTRODUCTION

Investigations into symmetrical easy standing have led to the expression of divergent views concerning the relation of the thigh to the pelvis at the hip joints, and the precise role of the hip musculature in the maintenance of such a posture. Åkerblom (1948) reviewed the literature and pointed out that a number of investigators considered that during 'comfortable standing' the hip joints were fully extended, the iliofemoral ligaments tense and the posture maintained with a minimum of muscular activity. On the other hand others, including more recently Steindler (1955), believed that in this posture the body was in a state of unstable equilbrium at the hip joints and that the erect position was maintained by contraction of the appropriate muscle groups. Åkerblom himself subscribed to the latter view, and pointed out that in previous investigations there had been a lack of uniformity and accurate definition of the posture investigated. Recently, Smith (1956), following investigations on the knee joint, has shown that the concept of the fully extended position of a synovial joint as being an absolute and limiting position is untenable, and that the actual position occupied by the joint is variable and directly related to the extending torque that is being applied to the joint.

Investigation of this problem using electromyography does not appear to have been carried out. If a very high amplification is used, this technique makes it possible to determine whether a muscle is active or not and it was decided therefore to employ this method in an investigation of the hip musculature during standing and during the performance of certain simple movements.

MATERIAL AND METHOD

A double-channel amplifier was used, together with a double-beam oscilloscope. The most important features of the apparatus were the low noise level of 2μV. and its high amplification ($\times 5 \times 10^6$) so that the minimum detectable potentials were of the order of 3μV. peak to peak. In order to reduce interference, the apparatus was surrounded by a screening cage. The amplifier had constant gain between 20 and 200 c./s. the response being reduced by one-half at 4 and 700 c./s. The potentials from the muscles were observed on the oscilloscope screen, and records were obtained by means of photographing the screen. Surface electrodes consisting of small silver cups were used and contact was maintained by suction. The electrode site was shaved, cleansed with spirit and scraped gently ten times with fine sandpaper. Cambridge electrode jelly was applied and gently rubbed into the skin.

The subjects used were eighteen males aged 18–42 years. The muscles investigated were (1) the iliopsoas, (2) the gluteus medius and minimus, and (3) the gluteus maximus. The sites for the attachment of the electrodes were chosen after preliminary

investigation. For the iliopsoas they were placed 4 cm. apart, parallel to and below the inguinal ligament, with the mid-point between the electrodes about 4 cm. below and medial to the anterior superior iliac spine. With the electrodes in this position the subject was asked to flex the thigh at the hip joint with the leg hanging vertically at the knee joint. Large potentials (0·5–1·0 mV.) were recorded during this movement. With the electrodes over the sartorius or rectus femoris no potentials were obtained during this movement.

When investigating gluteus maximus, the two electrodes were placed vertically about 4 cm. apart near the centre of the muscle mass. With the electrodes arranged in this way, large potentials (0·5–1·0 mV.) were recorded when the subject stepped on to a low stool. The muscle was felt to be contracted during this movement. The vertical disposition of the electrodes was used routinely, but in any individual the potentials obtained were similar with regard to their amplitude and number of peaks per second, however the electrodes were placed with respect to the main direction of the underlying muscle bundles.

With the subject abducting his thigh against resistance the site of the contracted gluteus medius and minimus muscles was determined by inspection and palpation. The electrodes were placed transversely 4 cm. apart over the area, about 7 cm. vertically above the tip of the greater trochanter. Large potentials (0·5–1·0 mV.) were recorded from this site during resisted abduction of the thigh and while standing on the recording limb.

The subject was asked to lie on a bed and recordings were taken from each site with the muscles relaxed. He was then asked to assume the symmetrical standing at ease position and, using the two channels simultaneously, recordings were taken from (1) iliopsoas and gluteus maximus, (2) iliopsoas and gluteus medius and minimus. In this posture the subject stood comfortably with the shod feet about 30 cm. apart, the feet turned outwards between 30° and 45° from the sagittal plane, the head erect and the hands lightly clasped behind the back.

The extensors of the thigh at the hip joint were investigated more fully, and simultaneous recordings were taken from the gluteus maximus and from the hamstrings during certain movements. When investigating the hamstrings one electrode was placed over the biceps femoris and one over the semitendinosus and semimembranosus 15–20 cm. above the level of the head of the fibula. At the beginning of each movement the subject was in the symmetrical standing at ease position and he returned to this position when the movement was completed. The movements investigated were (*a*) forward arm raising to 90°, (*b*) forward swaying at the ankle joints with the knees straight for approximately 5°, (*c*) slow bending at the hips to touch the toes with the knees straight. In order to illustrate the results obtained, two of the subjects repeated the movements on a number of occasions in time to a metronome, and subsequently synchronized cinematographic recordings of the movements were taken. The subjects were photographed against a background with vertical lines so that the position of the body relative to the vertical plane could be easily seen. Individual frames at intervals of 1 sec. were then taken from these films and matched with the appropriate part of the oscilloscope record. This method of recording was used since the screening cage made it impossible to film the subject and the oscilloscope screen simultaneously.

Records of amplifier noise and of calibration signals were taken at the beginning and end of the recording session for each individual in order to ensure that there was no variation in the amplifier throughout the experiment.

RESULTS

Relaxed muscle. Using the highest amplification available, the recordings over all the relaxed muscles studied were similar to those obtained in a previous investigation involving some relaxed leg and thigh muscles (Joseph, Nightingale & Williams, 1955). These records showed (*a*) a 'background' due to amplifier noise consisting of deflexions of 1·5–2 μV. amplitude occurring at 300–350 peaks/sec., (*b*) larger potentials of 3–7 μV. at 40–50 peaks/sec., and (*c*) deflexions of much longer duration, varying considerably in amplitude from the smallest detectable to 20 μV.

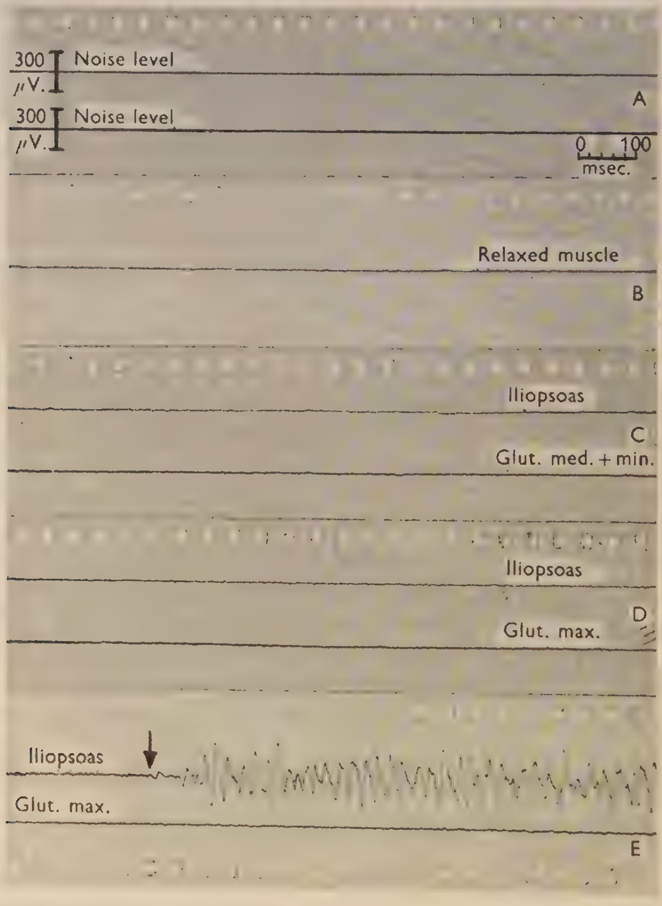

Fig. 1. A: Noise level, both channels; B: relaxed muscle; C: simultaneous recordings from iliopsoas (upper trace) and gluteus medius and minimus (lower trace) while standing at ease; D, E: simultaneous recording from iliopsoas (upper trace) and gluteus maximus (lower trace) while standing at ease. At arrow, subject flexed recording limb at hip.

Standing at ease. In all eighteen subjects the recordings from gluteus maximus and from gluteus medius and minimus, and in thirteen out of eighteen subjects the recordings from iliopsoas were similar to those obtained from the muscles when relaxed (Fig. 1). With the high amplification used, potentials from contracted

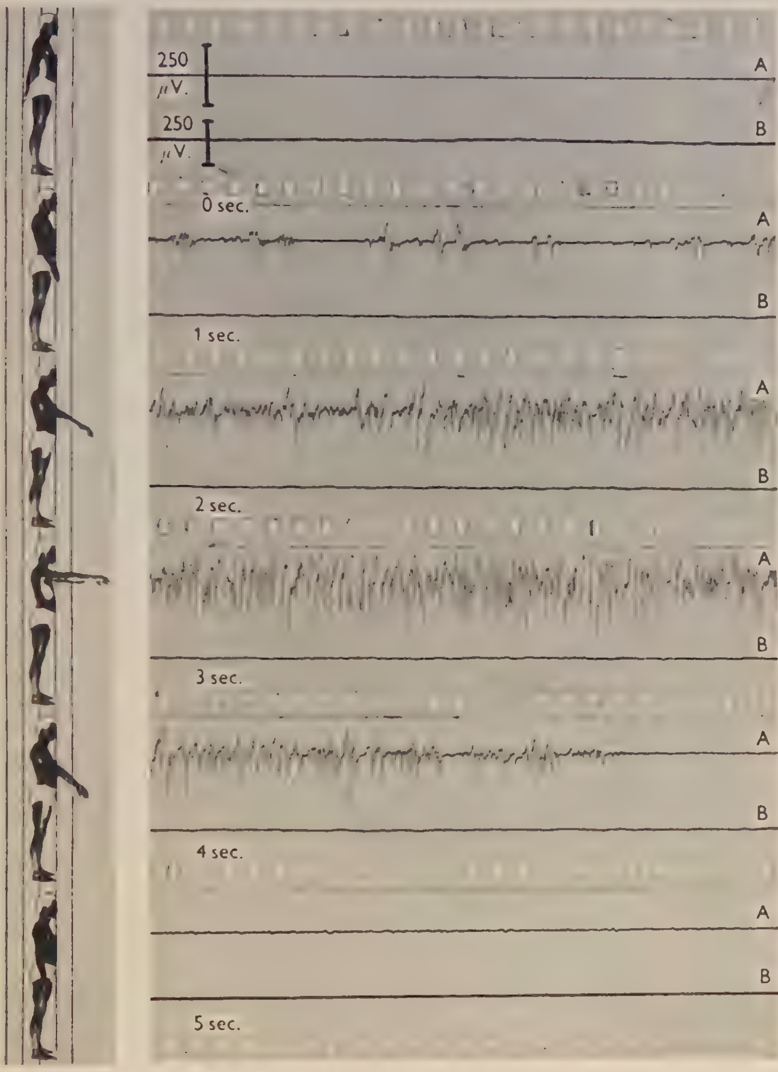

Fig. 2. Simultaneous recordings from (A), hamstrings, and (B), gluteus maximus during forward arm raising.

adjacent muscles, e.g. the pectineus in the case of the iliopsoas and the tensor fasciae latae in the case of the gluteus medius and minimus, could easily be picked up and it can be assumed that absence of potentials indicated absence of detectable activity in these muscles. In the remaining five subjects with the electrodes over iliopsoas, presistent potentials of approximately 100μV. were obtained. When these

subjects were asked to relax their abdominal muscles these potentials disappeared and a relaxed muscle picture was obtained. The thirteen subjects showing a relaxed muscle picture over iliopsoas during symmetrical standing at ease showed persistent potentials of about 100 μV. when asked to contract their abdominal muscles.

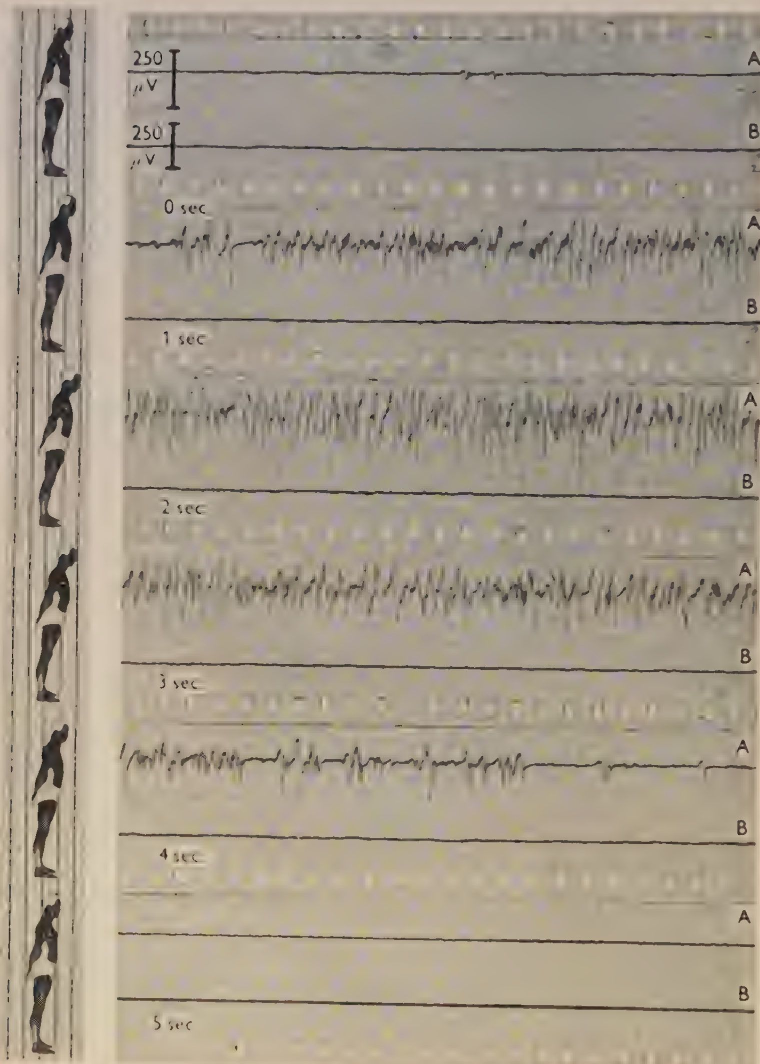

Fig. 3. Simultaneous recording from (A), hamstrings, and (B), gluteus maximus during forward swaying at the ankle joints.

Extensor activity at the hip joints. Simultaneous recordings were taken from the hamstrings and gluteus maximus throughout this part of the investigation, and the amplification was reduced considerably to facilitate recording of the relatively large potentials produced. During forward arm raising from the symmetrical standing at ease position, large potentials (about 500 μV.) were recorded from the hamstrings

when the arms were 5–10° from the vertical and were present throughout the rest of the movement. The gluteus maximus showed no evidence of electric activity (Fig. 2).

During forward swaying at the ankle joints, large potentials (about 500 μV.) appeared almost immediately from the hamstring group and persisted until the standing at ease position was regained. The recording from gluteus maximus again showed no evidence of electric activity (Fig. 3).

During toe touching with the knees straight, large potentials (about 500 μV.) were detected from the hamstrings almost immediately the movement commenced. These persisted throughout the downward and upward phases of the movement and disappeared only when the standing at ease position was regained. There was no demonstrable activity in gluteus maximus until the final stage of the downward phase of the movement when large potentials (about 400 μV.) were recorded. These persisted throughout the upward phase, and finally disappeared a little before the standing at ease position was again reached (Fig. 4).

DISCUSSION

The recordings over the relaxed muscles studied in this investigation were similar to those obtained over certain relaxed muscles of the leg and thigh studied previously. A detailed analysis of such records, and evidence suggesting that the deflexions recorded were not due to motor unit activity, were presented elsewhere (Joseph *et al.* 1955).

The present investigation has shown that in the eighteen subjects studied there was no postural activity in the iliopsoas, gluteus maximus, and gluteus medius and minimus during easy standing, and although the records were necessarily limited in length, they were representative of what was observed on the oscilloscope screen for periods of approximately 5 min. This is at variance with the commonly held view that a subject standing in the posture described is in a state of unstable equilibrium at the hip joints, and that accompanying the slight continual variations in posture that have been shown to occur (Hellebrandt, 1938), the upright position is maintained by activity in the appropriate muscle group. The intermittent type of activity, which is implied in this view, was not observed in any of the subjects studied during the present investigation.

In a careful radiological study of twenty-five subjects, it was shown (Åkerblom, 1948) that if an increased extending torque is applied experimentally, the hip joint could be extended 0–15° (mean 6°) beyond the position it occupied during symmetrical standing at ease. Furthermore, in ten subjects using simultaneous statography and radiography he showed that in the posture described, the line of body weight passed posterior to the hip joints (0 4 cm., mean 1·8 cm.). In view of these observations and the absence of postural activity demonstrated in the present investigation, it is thought that in the symmetrical standing posture, further extension at the hip is opposed by a passive mechanism. Smith (1956) has described the passive resistance to extension that occurs during the final stages of this movement at the knee joint, has analysed the factors contributing to the articular and non-articular components of the mechanism, and is of the opinion that such factors

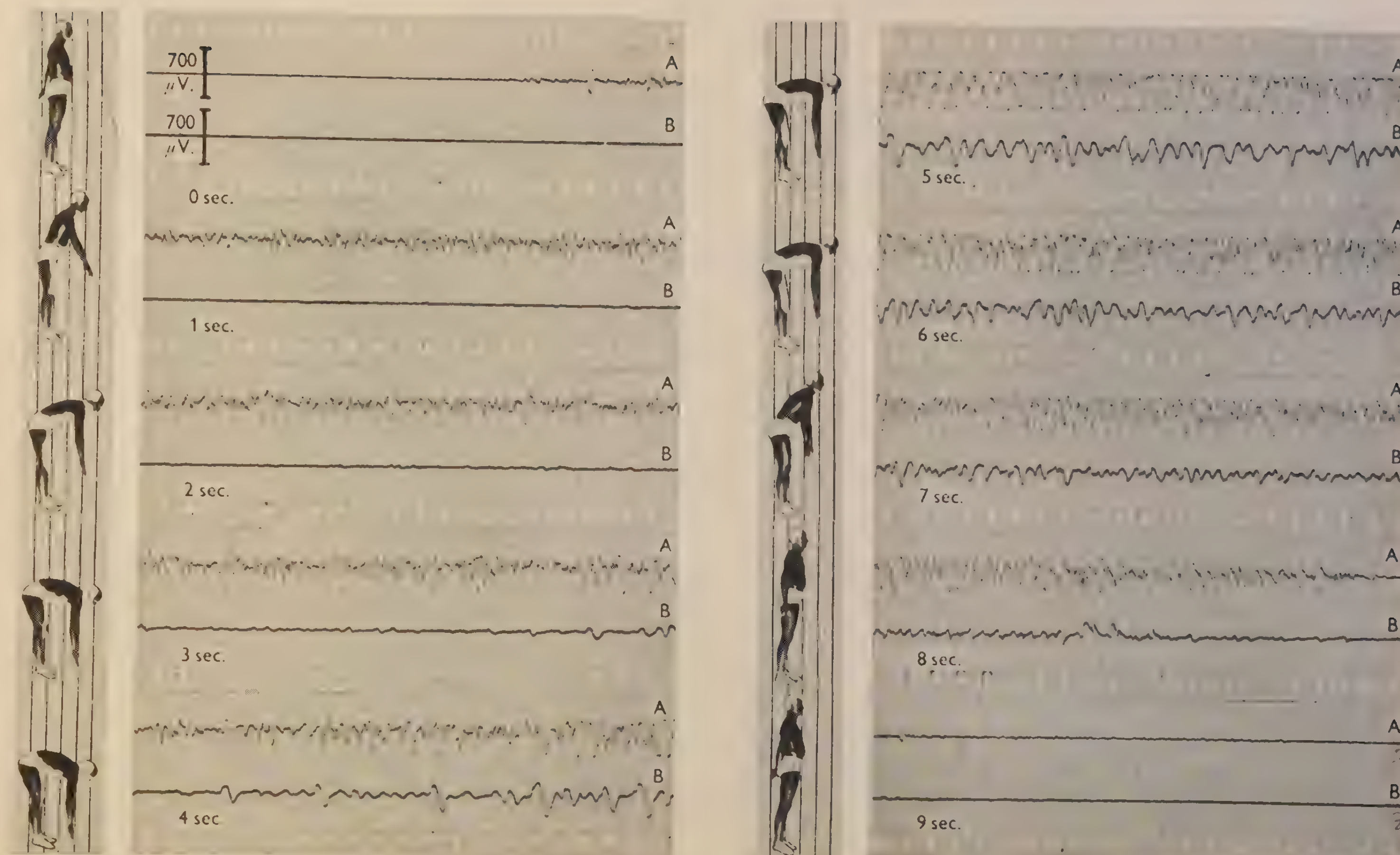

Fig. 4. Simultaneous recordings from (A), hamstrings, and (B), gluteus maximus during touching the toes with the knees straight.

must be operative to a varying extent in all synovial joints. In the symmetrical standing at ease position electromyographic studies have demonstrated absence of postural activity in the hip muscles studied in the present investigation, and also in the anterior and posterior thigh muscles in most subjects (Joseph & Nightingale, 1954). It is interesting to compare these observations with those of Hellebrandt, Brogdon & Tepper (1940), who used the method of closed circuit indirect calorimetry to determine the total energy expenditure of the body exposed to graded gravitational stress. It is stated that the increase in total energy expenditure during standing compared with that during recumbency is extremely small.

The experiments involving simple movements demonstrate the differential activity of the gluteus maximus and the hamstrings. The forward displacement of the line of body weight that occurs during forward arm raising, forward swaying and during the downward phase of toe touching, is accompanied by contraction of the hamstring muscles, and not gluteus maximus as suggested by Steindler (1955). It appears that during straightening up after touching the toes, more powerful extensor activity is required and simultaneous contraction of the gluteus maximus and hamstrings occurs during this movement. In order to explain the differences in the activity of the hamstrings and gluteus maximus in the upward and downward phases of the toe touching movement, these movements may be regarded as examples of positive and negative work respectively, as described by Abbot, Bigland & Ritchie (1952). They investigated the comparative cost in terms of oxygen consumption of positive and negative work and stated that positive work always costs more than negative work. The recordings obtained from the gluteus maximus and hamstrings during the present investigation support this finding and fit in with the concept of negative work as described by Hill (1951), who stated 'if the forces for lengthening and for shortening are to be made the same—as they must be if the external load is the same—the stimulus for the case of lengthening must be reduced either by exciting fewer fibres to activity or by reducing the frequency of the stimulation'.

SUMMARY

1. An electromyographic study has been made of the iliopsoas, gluteus medius and minimus, and gluteus maximus muscles whilst relaxed and during symmetrical easy standing in eighteen males aged 18–42 years.

2. The recordings obtained from the relaxed muscles were similar to those obtained in a previous investigation on certain relaxed muscles of the leg and thigh.

3. In the eighteen subjects studied there was no demonstrable postural activity in the iliopsoas, gluteus medius and minimus, and the gluteus maximus muscles during symmetrical standing at ease.

4. The differential activity of the hamstrings and the gluteus maximus during the performance of certain movements involving extensor activity at the hip joint has been investigated, and the significance of the results discussed.

We wish to thank Prof. R. Warwick for reading and criticizing the manuscript, and the Department of Medical Illustration, Guy's Hospital, for the figures.

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REVIEWS

Das Aschenbild tierischer Gewebe und Organe (*Micro-incineration of Animal Tissues and Organs*). By Erich Hintzsche. (Pp. iv + 140; 80 text-figures: 10×7 in.; D.M. 39.60 [approximately £3. 10*s.* 0*d.*].) Berlin: Springer Verlag. 1956.

The distribution of inorganic constituents within the tissues and their localization within the individual cells is of considerable importance for the understanding of the normal and pathological functions of organs. One approach to this problem is the method of micro-incineration, which was used for the first time by the French botanist, François Vincent Raspail, in 1833.

A good survey of the subject was given by Horning in his chapter in *Cytology and Cell Physiology* five years ago. Prof. Hintzsche of the Institute of Anatomy of the University of Bern, who himself has contributed to our knowledge in this field, has now summarized in this monograph the results obtained by micro-incineration of tissue sections. The contents of the book are arranged in a systematic order: a historical introduction is followed by a chapter on the technique and then each group of tissues and organs is dealt with in separate sections. A chapter on embryonic tissues includes the placenta and reports some aspects of Wislocki and Dempsey's extensive investigations. The book is well illustrated. The literature index fills 12 pages and appears to contain all relevant papers.

When reading through this survey of the field of micro-incineration two impressions are gained. First, there are the individual findings as, for example, the occurrence of silicon in tendon, umbilical cord and in the transitional epithelium of the urinary tract, or the occurrence of iron in the nuclei of the proximal convoluted tubules of the kidney. It seems that very little work has been done to place these facts into any functional context or to correlate them with pathological conditions.

Secondly, there are interesting observations of a more general character, such as those reported by Cathie and Davson (1937 and 1939) who found a correlation between the inorganic content of tumour cells and their sensitivity to irradiation, which might well be followed up. No comparison has so far been made of normal cells, with similar mitotic activities, as to their radiosensitivity and inorganic contents. The field of micro-incineration might be extended in this direction. However, the most fruitful line of pursuit in this subject appears to be a correlation of the observations made with the electron microscope with those of micro-incineration. Horning (1951) referred to this in describing the work of Scott and Parker (1939) on muscle fibres. Only Draper and Hodge (1940) seem to have made use of this combined technique in their work on the finer structure and localization of organic and inorganic constituents of myo-fibrils. Some pictures of incinerated cells, as for example those of mast-cells, look very similar to those obtained with the electron microscope. Hintzsche deals perhaps rather briefly with this promising extension of micro-incineration techniques in his otherwise full survey of this subject. W. JACOBSON

Studies on the Morphogenesis of the Brain in Struthio camelus. By Knud H. Krabbe. (Pp. 30; 11 plates; D.Cr. 25.) Copenhagen: Ejnar Monksgaard. 1956.

This beautifully printed contribution is in the nature of an appendix to the fifth volume of Dr Krabbe's extensive work on the descriptive morphogenesis of the vertebrate brain. That volume was concerned with the avian brain; the acquisition of six different stages of ostrich embryos has now enabled the author to add to his earlier account a description of the development of the brain in this species. The generally accepted primitive nature of the ostriches gives an added interest to the material. Apart from certain features in the paraphysis and the epiphysis, however, Dr Krabbe's investigation indicates that there is little in the developing ostrich brain suggesting special reptilian affinities. J. D. BOYD

Text-book of Human Anatomy. By J. D. Boyd, Sir Wilfred le Gros Clark, W. J. Hamilton, J. M. Yoffey, Sir Solly Zuckerman and the late A. B. Appleton. Edited by W. J. Hamilton. (Pp. xii+1022; 796 figures; £5.) London. Macmillan and Co. Ltd. 1956.

It is not the custom of this *Journal* to publish extensive reviews of text-books intended for students, but the appearance of a new major work is not a common event, and must clearly be of interest to all who are concerned in the teaching of human anatomy. It is important also because the outlook of anatomists and the orientation of the subject have changed very radically during the last 50 years, that is since the first editions of the books still in commonest use by British students were published. The present work is clearly intended to reflect these changes.

The book is considerably shorter than several others, and this lightening of the student's burden is to be welcomed. It has been achieved by the inclusion of less topographical detail, although one feels that more could have been omitted without serious loss. Some vocational aspects of the subject such as surface anatomy and radiological methods are dealt with very shortly and embryology is used only to a limited extent and where it throws light on particular aspects of structure, for example the disposition of the viscera. While the omission of anything will be regretted by someone, this difficult task of elimination has, on the whole, been well done and a balanced account of adult human structure has resulted.

If much has been omitted, there have been many additions long overdue in text-books of anatomy. The chapter on 'Growth' is one of these; it contains a surprising amount of important information not easy to find elsewhere, but might well have been increased a little in length by giving a somewhat fuller explanation of the significance of growth curves and of allometric growth. Many students may find the present account too condensed, and it is likely to lead to questions which their teachers, if not mathematically inclined, will have difficulty in answering. References to original literature, which are absent from all parts of the book, would have helped in matters of this kind. As one would expect, in all sections considerable emphasis is laid on microscopical structure and on function, a wholly admirable feature of the work. Some may think that this has meant only a substitution of physiological for anatomical detail; the hypophysis cerebri, for example, is dealt with in far greater detail than is usual in a text-book of anatomy and contains a considerable amount of information which, in the past, would have been found only in a text-book of physiology. There are other similar examples, and, though there may be differences of opinion how far such a process should go, there is no doubt it is going in the right direction. Apart from additions of this kind, all sections of the book have many excellent features. The illustrations for the digestive system are particularly good; the descriptions of the respiratory and vascular systems are clear and readable and the functional significance of the structures concerned is very well dealt with; the central nervous system is described most clearly in less than 130 pages and the difficult task of selection in this potentially vast field could hardly have been better done; in the description of the peripheral nervous system the introductory pages dealing with the general characteristics of the nerves are especially valuable. The section on bones, joints and muscles deals with these structures regionally rather than as independent systems, with obvious advantages for functional interpretation, although the student, approaching the subject for the first time, may find this method a little confusing. This is perhaps the least satisfactory part of the book, which may well be due to the untimely death of its author while it was still in preparation.

For a new text-book of this kind the Introduction is important since it is the first part which many students will read. A fuller account of the nature and aims of anatomy would be an advantage, and it is difficult to justify taking up half a page with figures 2 and 3 which are unlikely to be very clear to an elementary student. The short account of morphology and evolution (introducing the 'Locomotor System') is scrappy, and its illustration falls well below the standard of most of the book. This standard is in general high, but there are rather more errors, such as those in fig. 131, than one would have expected even in a

first edition. There are also occasional statements in the text which are misleading and possibly inaccurate such as the statement on p. 236 that in full extension the cruciate ligaments of the knee joint are parallel. It is disappointing that defects of this kind can be found so easily; probably none of them are of great importance and they can be corrected in future editions, but if much that is not essential is to be omitted, it is the more important that what remains should be accurate and of high quality.

These or other criticisms will occur to anyone who reads a book of this kind and who has the inevitable vested interests in one or other aspect of anatomy. Nevertheless, one can be grateful to the authors for the production of a book which goes far towards realizing the aims set out in the preface: to establish a scale of values in relation to topographical detail (involving the omission of much which is found in other text-books): to establish a closer correlation between the study of structure and function: and to establish and treat anatomy as 'an independent branch of biological science with problems and achievements of its own'. It is to be hoped that the book will be used widely and will have its effect in bringing greater enthusiasm and significance to the teaching of the oldest and in some ways the most fundamental of the biological sciences. F. GOLDBY

Normal Table of Xenopus laevis. Ed. P. O. NIEUWKOOP and J. FABER. (Pp. 243; 10 plates; $10\frac{1}{2}\times 7\frac{1}{2}$ in.; 22.75 guilders.) Amsterdam: North Holland Publishing Co.

The first major study on the development of *Xenopus laevis*, that of Edward J. Bles, appeared over half a century ago. Not for another forty years, in three papers by Weisz, were there described the numerous unique features of the anatomy of the larval stages of *Xenopus*. Weisz, however, did scant justice to the pioneer work of Bles.

By the 1940's the facility with which the adult *Xenopus* responds to mammalian gonadotropins was well known, and had been used as the basis of a pregnancy test for some years; embryologists also became aware of the advantages of an amphibian from which eggs could so readily be obtained throughout the year.

With the publication from the Hubrecht Laboratory at Utrecht of the *Normal Table of Xenopus laevis* the study of the development of this species acquires the crowning advantage of a survey in such detail that it will form the basis of further inquiries into any aspect of Anuran embryology. Future studies, based on this volume, may thus be expected gradually to reveal the importance of this project.

The work goes much beyond the normal form of a set of '*Normentafeln*'. The editors have collected together as many as twenty-eight separate inquiries on various aspects of development in *Xenopus* from a couple of dozen authors in nine countries. All of this data is combined into descriptions of sixty-six stages of development. The editors then collate the whole with previous descriptions of Anuran development and end with a general bibliography. The importance of the work is thus not confined to *Xenopus* alone.

The Hubrecht group have studied the development of their material at a temperature of 22–24° C., at which the late larva, termed by Weisz the 'third-form tadpole', is attained in under half the time which at 18° C. was noted by this author. Again, at Utrecht larvae at corresponding stages are larger than with Weisz. It appears that Dr Nieuwkoop and his colleagues have chosen optimal conditions for the development of *Xenopus* which may not always be attained elsewhere. To allow a volume of water not much less than 3 l. for each individual larva demands a considerable scale of operations. It is clear that the rate of development of the larva of *Xenopus* is extremely sensitive to the density of food particles in the environment.

However, this *caveat* apart, it is certain that the study of Anuran development will be immensely facilitated by this work of Dr Nieuwkoop and his colleagues, who must have devoted thereto long stretches of their time which they would otherwise have been able to give to still more of their distinguished original papers. We are deeply grateful to the Utrecht Laboratory. ARTHUR HUGHES

A Practical Manual of Medical and Biological Staining Techniques. By E. Gurr. 2nd ed. (Pp. 451 + xxv. £2. 0*s.* 0*d.*) London: Leonard Hill (Books) Ltd. 1956.

This is a second and expanded edition of a book originally based on a series of pamphlets on *Microscopic Staining Technique*. The author is the head of a well-known firm of suppliers of biological stains and reagents. It appears to be intended as a reference book of methods for laboratory workers, and matters of theory and general statement are therefore, according to the author, omitted for the sake of general clarity.

The work is divided into several sections, the two largest naturally being concerned with Animal Histology (normal and pathological) and Botanical Methods (normal and infected tissues). The staining procedures are arranged alphabetically in each section, sometimes under the name of the initial dye of a complex method, and sometimes under the name of the worker whose name is affixed to the method. This makes for confusion, and this confusion is not diminished by the inclusion of some methods which are of little practical value. In view of the efforts on standardization of stains which have been made, for many years now, especially by the Biological Stain Commission in the U.S.A., it is perplexing to find that the colour index of a stain is not mentioned in these methods, but only a stain name, which means nothing to the technician, accompanied by the advice that a certain trade brand should be employed. Where methods are concerned there is little critical evaluation between one procedure and another.

The section on Histochemistry is poor. No reference whatever is made to the demonstration of phosphatase and lipase which nowadays technicians are required to carry out in view of the research and diagnostic value of these methods.

One cannot recommend this book to the technician employed in research work in experimental laboratories because of the deficiencies noticed above. It may, however, be of some help to trainee technicians and to those whose work is concerned with the preparation of routine class material in botany and zoology. J. S. BAXTER

Anatomical Techniques. By D. H. Tompsett. Foreword by Sir Cecil Wakeley, Bt. (Pp. 240 + xvi; 83 illustrations, some in colour. 35*s.*) Edinburgh and London: E. and S. Livingstone Ltd. 1956.

The prosector to the Royal College of Surgeons of England has produced a book which has long been needed by all those who have to do with the preparation, mounting and display of material for teaching museums in Departments of Anatomy. When reading this work one is impressed with the essentially practical nature of the methods detailed therein and which are obviously the results of the experience over many years of an enthusiast for his work. Many technical details of great importance are included in these methods, details which some anatomists not acquainted with the ultimate desiderata of museum preparation might be inclined to ignore as time-wasting. Museum preparators will be grateful to Dr Tompsett for their inclusion.

The book is divided into four parts. The first deals with the preparation and mounting of human material and the second with the technique of illustration of anatomical dissections. The methods given here will find application in other than purely topographical work. The third and largest section deals with modern techniques of casting in synthetic resins and the preparation of models, while there is a final short section on the preparation and mounting of differentially stained slices of the brain. There is a short appendix listing various materials and where they can be obtained. Miss Jessie Dobson has provided a useful historical introduction to the book.

Mention must be made of the excellent paper on which the book is printed and the reproduction of the illustrations, especially those in colour. Leaders have been introduced to indicate essential features only, an example which might well be followed by those authors in whose books clarity of the original illustrations has been destroyed by excessive labelling.

One can recommend this book to anatomists, pathologists and all museum preparators as one which should be read and used. J. S. BAXTER

A QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS AND THEIR RELATIONSHIP TO AFFERENT FIBRES

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INTRODUCTION

Purkinje cell dendrites have two distinct, morphologically different, receptive regions each with its own specific afferents: the smooth branches, contacted by the climbing fibres, and the spiny branchlets, contacted by the parallel fibres (Cajal, 1895). The former are 'longitudinal axo-dendritic connexions' (Cajal, 1934, 1954), a type of synapse allowing an axon many contacts on the dendrites of a single neuron; the latter are 'cruciform axo-dendritic connexions of great length', a type of synapse permitting the dendrites of each neuron contact with a great number of axons and each axon contact with the dendrites of many neurons.

The recognition of two receptive regions in the Purkinje cell dendritic system resulted from Cajal's (1891) discovery of the peridendritic spines, which form a 'nap-like' covering on portions of the dendrites of certain neurons. Although their significance is unknown, Cajal considered the spines to be dendritic devices for increasing surface area and rendering the synapse more intimate. In view of his opinion, it is interesting that the spines are most abundant on the Purkinje cell branchlets, a perfect site of 'cruciform axo-dendritic connexions'.

Golgi (1894) noted that a Purkinje cell is easier to draw than to describe. The richness of its ramifications matches the richness of the related parallel fibre plexus (Pl. 1, figs. 1, 2, here only partially impregnated). Reflecting on the dendritic arborizations of the Purkinje cells, flattened in a plane at right angles to the dense stream of parallel fibres, and the extensive dendritic overlap of the Purkinje cells, Fox & Massopust (1953) concluded that this anatomical arrangement allows maximal convergence and maximal divergence in minimal space.

In the present study, an attempt was made to obtain some quantitative notion of this convergence and divergence by determining the density of the granule cells in the granular layer, the lengths of the parallel fibres, the spacing of the Purkinje cells, and the surface area of their spiny branchlets.

MATERIALS AND METHODS

The morphological observations reported here are based on a study of the extensive collection of Golgi preparations of the cerebellar cortex of the adult monkey (*Maccaca mulatta*), prepared by the modification of Fox, Ubeda-Purkiss, Ihrig & Biagioli (1951), available in this laboratory. The total length of a Purkinje cell's spiny branchlets was determined by the photographic cut-out method and the Chalkley

(1943) method. The latter gives the percentage of volume a particular element occupies in a histological section. The diameters of the branchlets and the spines were measured in sections under oil immersion with the ocular micrometer and on enlargements of oil-immersion photomicrographs.

Cell counts were made in formalin-fixed, cresyl-violet (B.D.H.) sections and the data obtained were corrected by the formula 'section thickness divided by section thickness plus nucleolar diameter' (Abercrombie, 1946; see also Agduhr, 1941; and Floderus, 1944). The actual thickness of the sections was determined under oil immersion by a series of measurements with the micrometer scale of the fine adjustment on a Zeiss Opton Microscope.

To ascertain the number of Purkinje cells per mm.^{2} of Purkinje cell layer, chamber and direct counts were employed. In the former the ocular micrometer was lined along the Purkinje cell layer and the number of cells with visible nucleoli within the length of the micrometer scale was noted. In the latter the cells with visible nucleoli were enumerated in predetermined lengths of the Purkinje cell layer, which were accurately determined with a map-measuring wheel on photographic enlargements of the same sections.

The number of granule cells per mm.^{3} of granular layer was determined by chamber and Chalkley counts. The average volume of a granule cell nucleus was calculated by direct measurements in sections and on photographic enlargements, and this value was used to convert the Chalkley ratio into absolute numbers.

The Golgi and Nissl materials used were fixed by perfusion of 10% formalin. The Golgi material was prepared far in advance of the present study; hence, the volume changes due to processing are unknown. No corrections for volume changes were made in the Nissl preparations, since the data obtained from both types of preparations were to be employed in common calculations. Thus, our results for the number of granule cells per mm.^{3} of granular layer and the Purkinje cells per mm.^{2} of Purkinje layer hold only for our fixed preparations.

OBSERVATIONS AND RESULTS

Purkinje cells in Golgi material. Cajal's (1911) description of the Purkinje cell dendritic pattern can easily be confirmed in the present Golgi material. Primary, secondary and tertiary smooth branches, successively slimming as they spread in a single plane and gradually ascend, form the basic framework and they give off (Pl. 2, fig. 8) the spine-laden, terminal branchlets. The branchlets, moderately branched and roughly the same in length, arise mostly from tertiary branches, but some of them, particularly the lowermost, take origin from secondary and primary branches.

In sharp contrast to the smooth branches, which never descend and never reach the pial surface, the inferior branchlets descend to a line running through the summits of the Purkinje cell bodies. The superior branchlets ascend to the pial surface, where some of them recurve inferiorly. Thus the branchlets and the parallel fibres are coextensive in distribution. Transversely, the branchlets extend beyond their smooth branches of origin and in intermediate regions span the spaces between the smooth branches, converting the dendritic system of each Purkinje cell into a flat,

sieve-like plate, which lies, without exception, broadside in the transverse direction of the folia. This orientation of the dendrites was known by the older anatomists, Stieda (1864), Obersteiner (1869) and Henle (1879), who revealed the smooth branches by the old carmine method. Strictly speaking, the smooth branches do form a fundamental plane, but Golgi preparations show clearly that this is not rigidly true for the branchlets. The latter are in planes two or three deep and some of them leave at slight angles to these planes. Yet owing to their thinness this does not noticeably increase the thickness of the dendritic formation. Cajal (1911) considered the staggered arrangement of the branchlets to be a further means of assuring contact with as many parallel fibres as possible.

Conspicuous oval spaces (Pl. 1, fig. 1, *s*) within the dendritic arborizations lodge blood vessels and stellate cell bodies (Cajal, 1911). We have never seen vessels here, but in some of our preparations cell bodies of unimpregnated stellate cells, tinged a chrome-yellow, are recognizable. We have also observed that a smooth branch always forms one boundary of these spaces. This is interesting in view of the recent disclosure of Scheibel & Scheibel (1954) that the climbing fibres, which run outward on the smooth branches, have processes contacting stellate cell bodies.

To appreciate the size relationships of Purkinje cell peridendritic spines, it is well to compare them with other structures and with the spines on other dendrites. For this reason all the photomicrographs on Pls. 2 and 3 were taken under oil immersion (obj. 100 × ; N.A. 132) and reproduced at the same magnification, except Pl. 2, fig. 7, which was taken under high power (obj. × 40; N.A. 0·75).

The spines emerge from the surface of the dendrites as straight, thread-like, protoplasmic processes, terminating in a spherule or knob. This is typical of their form on pyramidal cells of the cerebral cortex. They are shown on a branch of an apical dendrite (Pl. 2, fig. 3) and the dendritic shaft (Pl. 2, fig. 4) of a small pyramidal cell. On the dendrites of cells in the caudate nucleus (Pl. 2, fig. 5) they are more robust. The details of these processes can readily be observed under the high power of the microscope, but this resolution is inadequate for the proper study of the spines on the Purkinje cell branchlets. There they are too numerous and their thread-like processes are so short that the knob-ends appear to be on the surface of the branchlets. A careful study under oil immersion, however (Pl. 2, figs. 6, 8), reveals that these knob-ends are attached to the branchlets by a delicate process.

The knob-ends of the Purkinje cell dendritic spines are rather uniform in size, though this may not seem to be the case in Pl. 2, figs. 6 and 8, where the depth of field is extremely short and the spines are not all at the same level. Occasionally, in certain preparations, portions of the impregnated dendritic mass, for reasons unknown to us, have a crushed appearance and the knob-ends are dispersed like gunshot. In such fields (Pl. 2, fig. 7, high power), where the knob-ends are spread out in the same plane, their uniformity in size is striking.

Measurements of the spiny branchlets and the spines; density of the spines. In enlargements of oil-immersion photomicrographs of a number of different Purkinje cell dendrites a search was made for portions of spiny branchlets with spines in perfect focus, in order to measure the distance from the tip of one spine to the tip of the spine on the opposite side of the branchlet. Thirty such measurements reveal that this distance averages $2{\cdot}9\mu$. This was confirmed by direct ocular micrometer

measurements. Thus it is safe to assume that a cylindrical sheath 3μ in diameter will enclose a spiny branchlet.

The diameters of the spiny branchlets, without their spines, are 1μ, and the diameters of the knob-ends of the spines, although they are not perfect spheres, are approximately $0{\cdot}7\mu$.

From direct ocular micrometer measurements, under oil immersion, it was concluded there are from 14 to 16 spines per 10μ length of branchlet, or an average of 15 spines per 10μ length of branchlet.

Estimation of the total length of the spiny branchlets on a Purkinje cell. In estimating the length of the spiny branchlets by the photographic cut-out method a well-impregnated Purkinje cell (Pl. 1, fig. 1), with dendrites nearly parallel to the thick Golgi section, was photographed at four different focal planes, equidistant from each other, with the lens combination: Zeiss objective (25× ; N.A. 0·63); ocular 12·5×, the highest power that would include the entire dendritic spread in a single field. The profile depth of the dendritic plate, at any one place, is approximately 10μ, but since all parts of this cell are not perfectly parallel to the plane of focus, the distance from the plane of the first photograph to the plane of the last photograph is 16μ. A few branchlets in the lower right-hand corner are in focus in the uppermost plane, and a few branchlets in the upper left-hand corner are in focus in the lowermost plane. Enlargements (32 × 40 in.) were made and the branchlets sharply in focus cut out. The weight of the cut-outs, compared with the weight of a known area of similarly treated photographic paper, indicated that the excised spiny branchlets represent an area of $47{,}000\mu^2$, or a total length of $15{,}600\mu$ of branchlets 3μ in diameter. Another factor considered was the depth of field in the photographs. According to standard formulae for deriving depth of field (see Beadle, 1940; Brattgard, 1954) the lens combination used gives a depth of field of approximately 1μ. Allowing that the excisions may not always have been at the exact limits of sharp focus, calculations were made on the assumption that the cut-outs in each photograph represent $1{\cdot}5\mu$ of the total depth of 16μ. Therefore, in the four photographs the spiny branchlets were cut out for a depth of 6μ, or 37·5% of the total depth. The total length of the branchlets is then $41{,}700\mu$, a figure which correlates well with two Chalkley counts made on the spiny branchlets of the same cell by two different observers, working independently.

The first Chalkley count, referred to here as a random Chalkley count, was made under oil immersion with five pointers in the ocular. Arbitrarily the tip of one pointer was focused on a spiny branchlet and counts were made only at the tips of the other pointers, throughout all regions of the dendritic spread. The counts were periodically plotted on a graph, and when the curve levelled off, the hits were: 33% spiny branchlets, 7% smooth branches, and 60% unimpregnated space. The latter represents the space for parallel fibres, stellate cell bodies, and possibly portions of some of the elements sandwiched between the Purkinje cells: namely, the stellate cell dendrites, their axons, the dendrites of Golgi cells, and the Bergmann fibres. Since the Chalkley method gives only the percentage of volume a particular structure occupies, to compute the total length of spiny branchlets, it is necessary to know the volume of space in which the dendrites are spread. This was found to be $280 \times 310 \times 10\mu$, or $868{,}000\mu^3$; therefore, the volume of the spiny branchlets is $289{,}000\mu^3$.

This volume divided by the cross-sectional area of a tubule 3μ in diameter makes the length of the branchlets $40{,}900\mu$.

Since an accurate estimation of volume is important in calculations involving the Chalkley method, the second count was made in a predetermined volume ($300\times 400\times 10\mu$, or $1{,}200{,}000\mu^3$), which included and exceeded the volume in which the dendrites are disposed. To obtain equal samplings throughout this volume, the movements through all sectors were carefully controlled by the micrometers on the mechanical stage and the fine adjustment. These movements were systematic and supplied the necessary arbitrary element in this procedure, so that it was possible to make counts at the tips of all five pointers. The results were: spiny branchlets 23 %, smooth branches 6·2 %, and unimpregnated space 70·8 %. The volume of the branchlets then is $276{,}000\mu^3$ and their length is $39{,}400\mu$.

The average of these three determinations is $40{,}700\mu$; thus in subsequent calculations all the branchlets were considered as one branchlet of this length. The problem of branching was disregarded because the surface area consumed by branching is more than regained by the free-ends of the branchlets; moreover, we knew of no way of gathering data to make accurate allowances for this factor.

Such a branchlet, cylindrical in shape and 1μ in diameter without spines, has a surface area of $128{,}000\mu^2$, and if there are 15 spines per 10μ length of branchlet, it has a total of 61,000 spines. If the knob-end of each spine is a spherule $0{\cdot}7\mu$ in diameter, each knob-end has a surface area of $1{\cdot}5\mu^2$ and the spines contribute a surface area of $93{,}900\mu^2$, making the combined surface area of the branchlets and the spines $222{,}000\mu^2$. The delicate filament attaching each knob-end of a spine to a branchlet was not considered; its extreme fineness renders measurements impractical, and any loss of surface area due to its attachment at the branchlet and at the knob-end of the spine must be more than compensated for by the surface of the filament itself.

The density of the Purkinje cell bodies in the Purkinje layer

Henle (1879) directed attention to the spacing of the Purkinje cell bodies and observed in many places that they are separated by less than the diameter of a single cell, and that elsewhere the separation may be three or four times this distance. In the present study we could find no significant difference between Purkinje cell counts made in mid-line sagittal sections and in sagittal sections through the mid-lateral extent of the cerebellum, but we did find, in agreement with Henle (1879) and Obersteiner (1888, 1890), that the cells are usually closer together at the summits of the folia than they are at the depths of the sulci. Obersteiner attributed this to the proportional amount of free surface exposed, '...since each cell has to provide for an equal segment of the cortical surface..., on the convexity, the superficial area is greater than it is in the concavity, the number of Purkinje cells varies accordingly'. In sections of the cerebellum the folia differ in shape and size and it is difficult to decide where to start and stop counting at the convexities and concavities of the folia. But in counts made it was estimated that the Purkinje cell ratio in the two regions is approximately four to three, respectively.

The Purkinje cell bodies form a single sheet of cells at the molecular-granular layer junction. To determine their density per mm.² of this cellular sheet, counts

were made covering all portions of the Purkinje cell layer, i.e. in the fundi, banks and summits of the folia. The results were: chamber counts, 530 Purkinje cells per mm.^{2} (59 cells counted); direct count, 510 Purkinje cells per mm.^{2} (263 cells counted). Weighing these results in proportion to the cells counted and rounding the figure there are 510 Purkinje cells per mm.^{2} of Purkinje layer.

The granule cells in Nissl and Golgi preparations. The granule cells are prodigious in numbers. Unlike other neurons, their nuclei (Pl. 3, fig. 14) contain deeply staining clumps of chromatin, and their cytoplasm is devoid of recognizable Nissl substance. But comparing an impregnated cell (Pl. 3, fig. 13) with the nuclei (Pl. 3, fig. 14) gives some indication of the perikaryon's extent.

Three to six slender dendrites stem from the nearly spherical cell body and terminate in short digit-like branchlets, which sprout small protoplasmic processes (Pl. 3, fig. 13) comparable in size and structure to the dendritic spines on the cells of the caudate nucleus (Pl. 2, fig. 5). Occasionally a dendrite divides; then each resulting branch bears a set of digitiform endings.

The digitiform endings come into relationship with the mossy fibre rosettes; several or more of them from as many different granule cells converge on and contact a single rosette. We have observed a rosette articulating with the digits from three granule cells, but undoubtedly larger rosettes accommodate the digits of even more granule cells.

The sites of these complex synapses are the cerebellar islands, the clear spaces (Pl. 3, fig. 14, *i*) in Nissl preparations; here Golgi cell axons, as well as mossy fibre rosettes and granule cell digits, are involved in an intricate relationship. The granule cell bodies, clustering close together, are aggregated around the cerebellar islands. Each island, therefore, is in position to have granule cell dendrites converging on it from all directions, and each cluster of granule cell bodies, with dendrites protruding in all directions, is in position to radiate dendrites to several or more nearby cerebellar islands. The lengths of the dendrites supply some clue to the spacing of the cerebellar islands, since the length of a dendrite is dependent on the position of the cell body in the cluster and the distance it must travel to reach a rosette. We have reason to believe that a granule cell may send one of its dendrites to a more distant island, for we have observed on a few occasions granule cell dendrites of unusual length, six to seven times as long as the dendrite shown in Pl. 3, fig. 13.

The axons of the granule cells emerge either from the cell body or from a dendrite. If the latter is the origin, it is usually an ascending dendrite and not a horizontal or descending dendrite, which illustrates one of Cajal's (1909) generalities on the nervous system, the 'law of economy of matter'. The axons ascend to the molecular layer where they bifurcate T-shape (Pl. 3, figs. 10, 12) and run parallel to the long axis of the folium; hence, the designation (Cajal, 1911) 'parallel fibres'. In general, cells low in the granular layer give rise to inferior parallel fibres, and cells high in the granular layer give rise to superior parallel fibres (Cajal, 1911). This can easily be confirmed in the present material.

According to Cajal (1911) the parallel fibres vary in diameter between 0·5 and 0·2μ, and he observed (Cajal, 1903) that only the parallel fibres in the lower third of the molecular layer stain in reduced silver preparations. The refractoriness of the superior parallel fibres to reduced silver suggested to Cajal (1926) that they were

thinner than the inferior fibres. Fox, Ubeda-Purkiss & Massopust (1950) demonstrated that the calibre of the parallel fibres gradually decreases from below upwards in the molecular layer. Pl. 3, fig. 12, shows an inferior parallel fibre slightly more than 1μ in diameter, and Pl. 3, fig. 10, shows a superior parallel fibre slightly less than $0{\cdot}2\mu$ in diameter. Both fibres are from the same field and, in the interval between them (not shown here), there are two other bifurcating granule cell axons, intermediate in size.

The parallel fibres maintain the plane adopted at the bifurcation, and their general direction is not altered by the short flexuosities imposed upon them by the innumerable dendritic processes through which they thread their way. These flexuosities are more pronounced in sections parallel to the molecular surface (Pl. 2, fig. 9) than they are in sections perpendicular to the molecular layer (Pl. 3, figs. 10–12).

The existence of synaptic endings, described by Estable (1923), on the parallel fibres in the form of boutons and warty rosettes can be confirmed in our preparations (Pl. 2, fig. 9; Pl. 3, figs. 10, 11). In addition, we have observed hook-like endings (Pl. 2, fig. 9, left side). Since they are apparent only in sections cut parallel to the surface of the molecular layer, we are inclined to believe that the warty rosettes are nothing more than hook-like endings cut in such a fashion as not to reveal their true form. For example, if the parallel fibre (Pl. 3, fig. 10) were viewed in a section parallel to the surface of the molecular layer, the warty endings seen on the left side of this figure might well have the appearance of the hook-like endings seen on the left side (Pl. 2, fig. 9).

We have never observed synaptic endings at or near the parallel fibres' T-shape bifurcation. Note the absence of endings on the fibre (Pl. 3, fig. 12). The first synaptic ending seen on the direct continuation of this fibre (Pl. 3, fig. 11) is 90μ from the point of bifurcation. However, we hesitate to draw conclusions concerning the distribution of the endings because one never knows how complete impregnations are in Golgi material.

The farthest we have traced a parallel fibre from its point of bifurcation is 1 mm. in each direction, but we were uncertain whether the actual terminations had been reached. Another observation has convinced us that even the heaviest, and presumably the longest, parallel fibres do not go much beyond this in the adult monkey. Carefully following, under oil immersion, one arm of a heavy-calibre inferior parallel fibre, similar to the fibre in Pl. 3, figs. 11, 12, we were able to observe that it maintained its usual thickness and displayed warty rosettes and sessile boutons for a stretch 90–950μ from the bifurcation point; then it thinned appreciably and 1500μ from the bifurcation point it was barely perceptible, leading us to believe its termination had been reached. Assuming the opposite arm of this fibre, cut off in section, is equally extensive, the combined length of the arms of this fibre is around 3 mm.

We feel rather certain that the maximal length of the parallel fibres is approximately 3 mm., and that some fibres are at least 2 mm., but we are not so certain of the minimal length. It is not unusual to follow one arm of a superor, thin, parallel fibre from 400 to 500μ, which would indicate the range of the two arms is approximately 1 mm. or slightly less.

To determine the average length of the parallel fibres from the data obtained with

the Golgi method is impossible. For this reason calculations were made on the basis that the average length is 2 mm. or 1·5 mm. However, we believe the average length is nearer 1·5 mm. than 2 mm., because there are many more of the shorter superior parallel fibres.

Ratio of the granular layer to the molecular layer; average thickness of the granular layer; density of the granule cells. The ratio of the cross-sectional area of the granular layer to the cross-sectional area of the molecular layer was determined by Chalkley counts in a series of sagittal sections. Representative regions were surveyed from the mid-line to the lateral extent of the cerebellum and the ratio found was: molecular layer, 1·5; granular layer, 1.

The granular layer is thicker at the summits than at the troughs of the folia; undoubtedly this reflects the fact that there are more Purkinje cells at the convexities of the folia. Its average thickness, 0·2 mm. in cell-stained sections, was calculated by measuring its area on photographic enlargements and dividing by the length of the Purkinje cell layer. Direct measurements along the straight part of the folia indicate this figure to be of the proper magnitude.

The number of granule cells per mm.^{3} of granular layer is: chamber counts—2·35 million granule cells per mm.^{3} (approximately 2000 cells counted); Chalkley counts—2·44 million granule cells per mm.^{3} (approximately 2000 cells counted), giving a weighted average of 2·4 million granule cells per mm.^{3} of granular layer.

Beneath each mm.^{2} of the Purkinje cell layer (beneath every 510 Purkinje cells) there are (2·4 million $\times$ 0·2 mm.) 480,000 granule cells, or 960 granule cells for every Purkinje cell.

The convergence and divergence of the parallel fibres. How many parallel fibres come within the dendritic spread of a Purkinje cell such as shown (Pl. 1, fig. 1)? There are on an average 480,000 granule cells below each mm.^{2} of Purkinje cell layer, and the cell in question has a transverse dendritic spread of 0·31 mm. The number of fibres that could occupy the same area as these dendrites should then be the product of 480,000 $\times$ 0·31 $\times$ the average length of the parallel fibres. If the latter is 1·5 mm., the number of fibres is 223,000 and if it is 2 mm., the number is 297,000.

Calculations can also be made from another point of view. The ratio of the granular layer to the molecular layer is 1 to 1·5. Assuming the elements of the two layers are related to each other in the same ratio, the area occupied by the dendrites (Pl. 1, fig. 1) ($310 \times 280\mu$ or $86{,}800\mu^2$) reduced by two-thirds and multiplied by the average length of the parallel fibres should give the volume of the granular layer with granule cells related to this area of dendrites. Calculated this way, if the average length is 1·5 mm., there are 208,000 fibres, and if it is 2 mm., there are 278,000 fibres.

Since there are 510 Purkinje cells in a mm.^{2} of the Purkinje cell layer, and since the transverse spread of a Purkinje cell's dendrites is at least 0·3 mm., a single parallel fibre coursing through the molecular layer can contact the branchlets of Purkinje cells in a patch 0·3 mm. in width. Parallel fibres 3, 2 and 1·5 mm. in length can diverge to 460, to 310 and to 230 Purkinje cells respectively.

DISCUSSION

The spines, also known as thorns or gemmules, have been neglected in recent times. They have been considered artefacts produced by the Golgi method (Kölliker, 1896; Meyer, 1895, 1896), endfeet implanted on the dendrites (Held, 1897; Bodian, 1940), and dendritic collaterals (Cajal, 1909). Hill (1897) concluded that they belong both to the dendrites and to the afferent fibres and logically added: 'If my interpretation of the thorns is the right one, we are brought back to the theory of Gerlach....'

The spines have been stained by methods other than the Golgi technique. Cajal (1896) and Turner (1901) revealed them by modifications of the Ehrlich method, and Hill (1896) observed that they are distinct in sections, deeply stained with haematoxylin and then broken up with needles in glycerine. Hatai (1903) devised a procedure in which small pieces of nerve tissue, simultaneously fixed and stained in a mixture of acid fuchsin, formalin, acetic acid and picric acid, renders the ordinarily achromatic portions of axons and dendrites visible. The gemmules, according to him, are merely local extensions of the dendritic ground substance, and internal staining differences distinguish them from the axonic terminals.

Cajal (1909) contended that the constancy of the spines in the same locations on the same neurons proves their pre-existence, and he argued that the impossibility of staining them by neurofibrillar methods, which stain the endfeet, indicates that the spines and the endfeet are not identical structures. In his last monograph Cajal (1934, 1954) reiterated his strong conviction that the spines are dendritic collaterals and emphasized that though always thin proximally, they are enlarged distally into a bulb or knob, while the reverse is true of the endfeet.

Although there is no unanimity as to what the spines are, we believe they are dendritic collaterals occurring in regions where the dendrites meet oblique or cruciform afferent fibres. If, on the other hand, they are synaptic endings, it is possible to determine the number of parallel fibres on a Purkinje cell's branchlets by more or less direct methods. Our estimate, then, of the number of spines, 61,000, would be the number of parallel fibres converging on a Purkinje cell. But actually the structure of the parallel fibre endings destined for the branchlets is not known. The spines (Pl. 2, figs. 6, 8) are uniform in size and smaller than the hook-like, sessile bouton and warty rosette, parallel fibre endings (Pl. 2, fig. 9; Pl. 3, figs. 10, 11), and this might be considered good evidence that the spines are not synaptic terminals. But lest this be construed as conclusive proof, it is well to remember that parallel fibres synapse also on the dendrites of Golgi cells and the dendrites of the various stellate cells. Therefore, the possibility exists that the endings specifically for the Purkinje cell branchlets may not be revealed in our preparations.

In Golgi preparations (Pl. 1, figs. 1, 2) the number of parallel fibres converging on a Purkinje cell's branchlets is enormous. To count these fibres directly in a segment through the width of the molecular layer, however, is impossible; reduced silver techniques stain only the large inferior parallel fibres, and Golgi preparations, with their sporadic staining, are not good for quantitative analysis. Thus it was necessary to take recourse to indirect methods in arriving at some numerical notion of the convergence on a Purkinje cell's branchlets.

Incidentally, when two different types of calculations were made on the basis that

the parallel fibres' average length is 1·5 mm., the number of fibres (223,000 and 208,000) passing through the dendritic spread of the Purkinje cell approximates the 222,000 μ^2 estimated for the surface areas of the same cell's branchlets and spines. How many of these fibres contact spiny branchlets is impossible to say, but the staggering of the branchlets suggests they are set to synapse with as many fibres as possible. The calculated total length of the branchlets (40,700 μ) emphasizes that the branchlets are arranged in several planes, for if these same branchlets, 3 μ in diameter, were in lengths 280 μ (i.e. height of the dendritic system) and arranged side by side in a single plane, they would extend for 430 μ, a distance considerably greater than the dendritic spread of the cell. Not many fibres, it would seem, could work their way through this system, and miss the branchlets. Yet (considering the smallest number calculated, 208,000) if only half or one-third make contact, there would be in the first instance 104,000 and in the second instance 69,000 parallel fibres converging on a single Purkinje cell. The latter figure is close to the number estimated for the spines. Incidentally, Lorente de Nó (1934), who was undecided whether the spines are part of the dendrites or endings impregnated without their fibres, had no doubt each spine is a synapse. Certainly if the spines are dendritic collaterals for increasing receptive surface and rendering the synapse more intimate, there must be at least one synapse for each spine and the number of spines represents the least possible number of endings on the spiny branchlet.

Why thousands of fibres are in touch with a Purkinje cell's terminal spiny branchlets is unknown, but it may be meaningful in the light of the recent disclosure that dendrites have properties different from those of the cell bodies and axons. Clare & Bishop (1955 *a*, *b*), studying intercortical paths activating only the apical dendrites of the cortical pyramids, have obtained evidence indicating that dendrites in the cerebral cortex do not behave as all-or-nothing conductors. Repetitive stimulation maintains them in a persistent state of negativity. They have no absolutely refractory period, and the duration of their response is much longer than that of the cell bodies and axons. They modulate the activity produced in cell bodies by afferent stimulation of cell bodies and they are capable of sustaining local activity at a stimulated region without propagating impulses to the cell body or axon. Interestingly, these disclosures were made in paths synapsing like the parallel fibres on Purkinje cell branchlets—both are exclusively axodendritic and both end on terminal spiny branchlets.

Is the fantastic number of parallel fibres on Purkinje cell branchlets responsible for the fast activity of the cerebellar cortex? Adrian (1935) and Dow (1938) have shown that the electrical discharge frequency of the cerebellar cortex is one of the fastest in the central nervous system. Snider & Eldred (1948), by isolation experiments, demonstrated that this rapid activity results from intrinsic rather than extrinsic mechanisms. Brookhart, Moruzzi & Snider (1951), with microelectrode recordings of unit activity, have found this activity to originate from structures in the Purkinje cell and/or granule cell layers. If, for the moment, this activity is speculatively assigned to the Purkinje cells, say as a result of their rich connexions with parallel fibres, the silence Brookhart *el al.* encountered in the molecular layer, while probing with microelectrodes for unit spike activity, may be explained by the dendritic properties Clare & Bishop disclosed.

The estimates that parallel fibres 3 mm. in length, 2 mm. in length and 1·5 mm. in length diverge to 460, to 310 and to 230 Purkinje cells respectively, presume that a fibre synapses with all the branchlets protruding in its pathway from the greatest number of cells possible. Regarding the maximal lengths of the parallel fibres, it is interesting that Dow (1949), recording potentials along the axis of the parallel fibres, was unable to obtain records when the stimulating and pick-up electrodes were separated by a distance greater than 5 mm. There is, perhaps, no great discrepancy between our results and those of Dow, if the technical difficulty of having stimulating and recording electrodes so close together and the possibility of stimulus spread are taken into consideration.

Convergence and divergence are responsible for central overlap in the nervous system. Sherrington (1929) used central overlap to explain the phenomena of occlusion in motor reflexes and, undoubtedly, much remains to be explained by central overlap. Lorente de Nó (1934), observing what he called the 'partially shifted' overlap in all centres studied, concluded it must be highly significant and, speculating on its possible physiological role, elaborated on its participation in the projection to and the parcellation of functional fields.

It is difficult to conceive of an arrangement better suited for convergence and divergence than the overlapping, sieve-like plates of the Purkinje cell dendrites flattened in the dense stream of the parallel fibres. It is an architecture ideal for central overlap. In the folia this overlap is fixed transversely by the width of the Purkinje cell dendrites and longitudinally by the lengths of the parallel fibres. Transversely, cell bodies separated from each other by a distance of 300μ easily share in common some fraction of their synaptic pools, while longitudinally, with the composition of the parallel fibre plexus ever changing by the addition and termination of fibres of varying lengths, there is from cell to cell an exquisite gradation in the fractionation of synaptic pools. Cells separated from each other by a distance of 3 mm. in the longitudinal direction share some fringe of fibres in common.

The axons of Golgi cells and mossy fibres converge on granule cell dendrites. Cajal (1911) and Scheibel & Scheibel (1954) have shown that a single mossy fibre sends branches to adjacent folia. Within the granular layer these branches ramify and each ramification has a series of rosettes. Since there are three or more granule cells contacting each rosette, it is clear that a single mossy fibre diverges to a large number of granule cells. But, on the other hand, each granule cell, having three to six dendrites, is in position to have converging on it as many different mossy fibres as it has digitiform endings. Thus there is convergence on a neuron as small as a granule cell. Single unit recordings in the brain stem reticular formation (Scheibel, Scheibel, Mollica & Moruzzi, 1955), in all the nuclei of the amygdaloid complex (Machne & Segundo, 1956) and in the lenticular nucleus and the claustrum (Segundo & Machne, 1956) have demonstrated that there is widespread convergence of impulses on individual neurons, even from the opposite ends of the central nervous system. To record from a single granule cell would certainly be a technical feat, but it would be interesting to know, for example, in the anterior lobe of the cerebellum, where there is some possibility of spino-cerebellar and cortico-pontocerebellar convergence, if individual granule cells may be stimulated from such widely separated sources.

SUMMARY

The primary, secondary and tertiary smooth dendrites of the Purkinje cells sprout terminal spiny branchlets, which are commensurate and coextensive with the parallel fibre plexus generated by the granule cell axons. The parallel fibres form 'cruciform axo-dendritic connexions' with the Purkinje cell spiny branchlets. The overlapping of the Purkinje cell dendrites and the rigid orientation of the Purkinje cells and the parallel fibres results in an arrangement in which there is maximal convergence and maximal divergence in minimal space. In the present study of the cerebellar cortex in the adult monkey (*Maccaca mulatta*) an attempt was made to derive some quantitative concepts of this divergence and convergence from observations and measurements in Golgi preparations and from cell counts and measurements in cresyl-violet preparations.

In our fixed preparations there are an average of 510 Purkinje cells per mm.^{2} of Purkinje cell layer and 2·4 million granule cells per mm.^{3} of granular layer. The ratio of the cross-sectional area of the molecular layer to the cross-sectional area of the granular layer is 1·5 to 1 respectively. The average thickness of the granular layer is 0·2 mm.

The parallel fibres display hook-like, sessile bouton and warty rosette endings, which are larger than the spines on the Purkinje cell branchlets. The parallel fibres decrease in calibre and in length from below upwards in the molecular layer. The inferior parallel fibres are approximately 1μ in diameter and 3 mm. in length and the superior parallel fibres are approximately $0{\cdot}2\mu$ in diameter and 1 mm. or less in length. The thin-calibre parallel fibres are more numerous than the thick-calibre parallel fibres.

The average of three determinations for the total length of the spiny branchlets on a Purkinje cell is $40{,}700\mu$. This calculation emphasizes that the branchlets are staggered in several or more planes. These branchlets have a total of 61,000 spines. The combined surface area of the branchlets and the spines is $222{,}000\mu^2$.

It is not known for certain whether the spines are synaptic endings on the dendrites or dendritic devices for increasing synaptic surface and rendering the synapse more intimate. In either case, however, their number is a good index of the number of fibres converging on the Purkinje cell branchlets.

There are more than enough fibres passing through the area of the dendritic spread of a Purkinje cell to account for the number of spines. It was estimated (based on the average thickness of the granular layer) that if the average length of the parallel fibres is 1·5 mm. or 2 mm., there are 223,000 fibres or 297,000 fibres, respectively, passing through the area of dendritic spread. It was also estimated (based on the ratio of the molecular layer to the granular layer) that if the average length of the parallel fibres is 1·5 mm. or 2 mm., there are 208,000 fibres or 278,000 fibres, respectively, passing through the dendritic spread of the Purkinje cell.

The enormous convergence on a Purkinje cell's spiny branchlets was discussed in the light of the recent disclosure that dendrites may not behave all-or-nothing.

It was estimated that parallel fibres 3, 2 and 1·5 mm. in length can diverge to 460, to 310 and to 230 Purkinje cells, respectively.

The central overlap in this system is limited by the transverse spread of the

Purkinje cell dendrites and by the lengths of the parallel fibres. Purkinje cells separated from each other by 300μ in the transverse direction of the folia and by 3 mm. in the longitudinal direction of the folia can share in common some fringe of parallel fibres. The closer Purkinje cells are to each other in both directions, the more parallel fibres they share in common.

There is convergence on a neuron even as small as a granule cell, for each granule cell contacts as many mossy fibres as it has digitiform endings.

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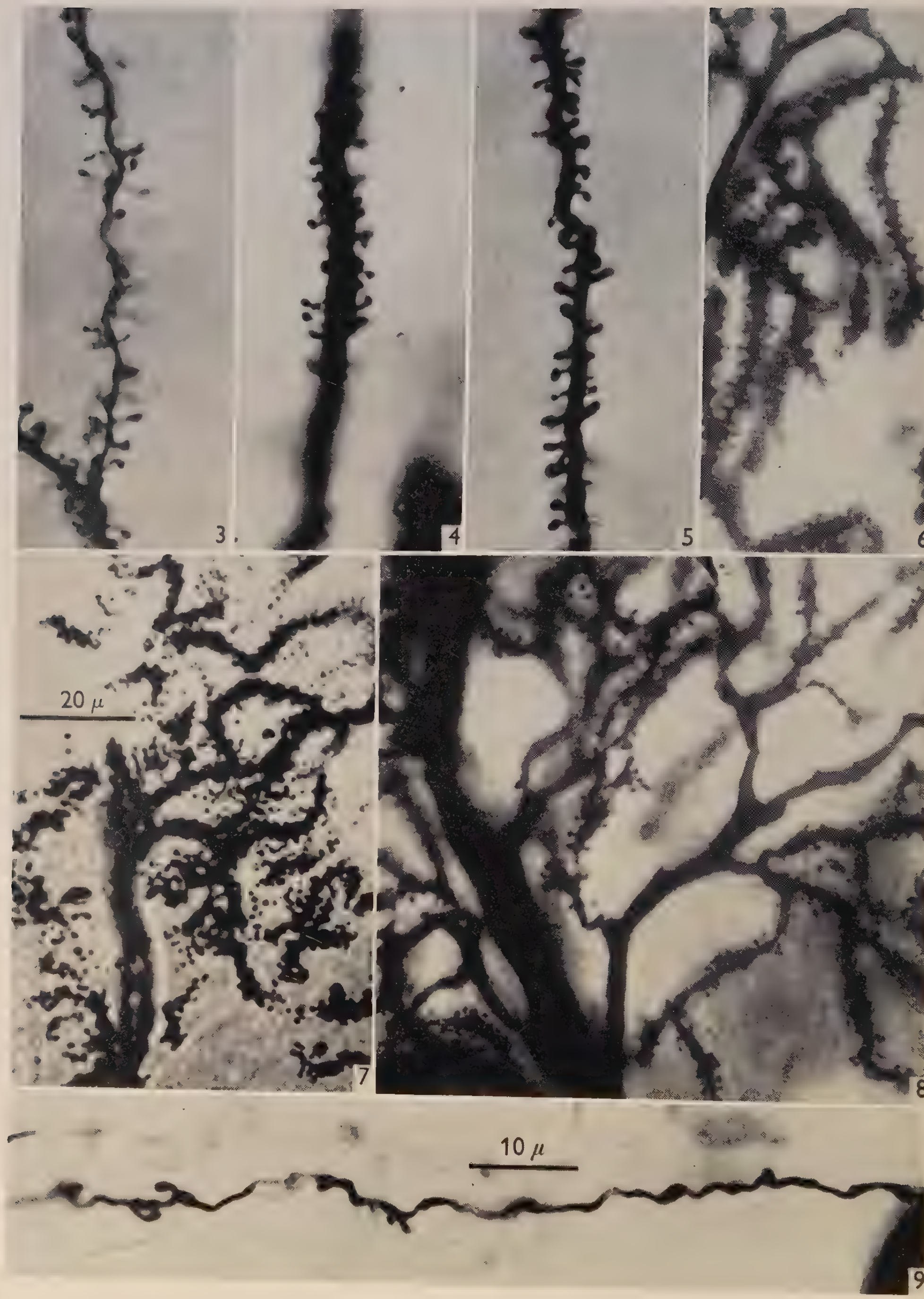
FOX AND BARNARD—QUANTITATIVE STUDY OF THE PURKINJE CELL DENDRITIC BRANCHLETS

(*Facing p.* 312)



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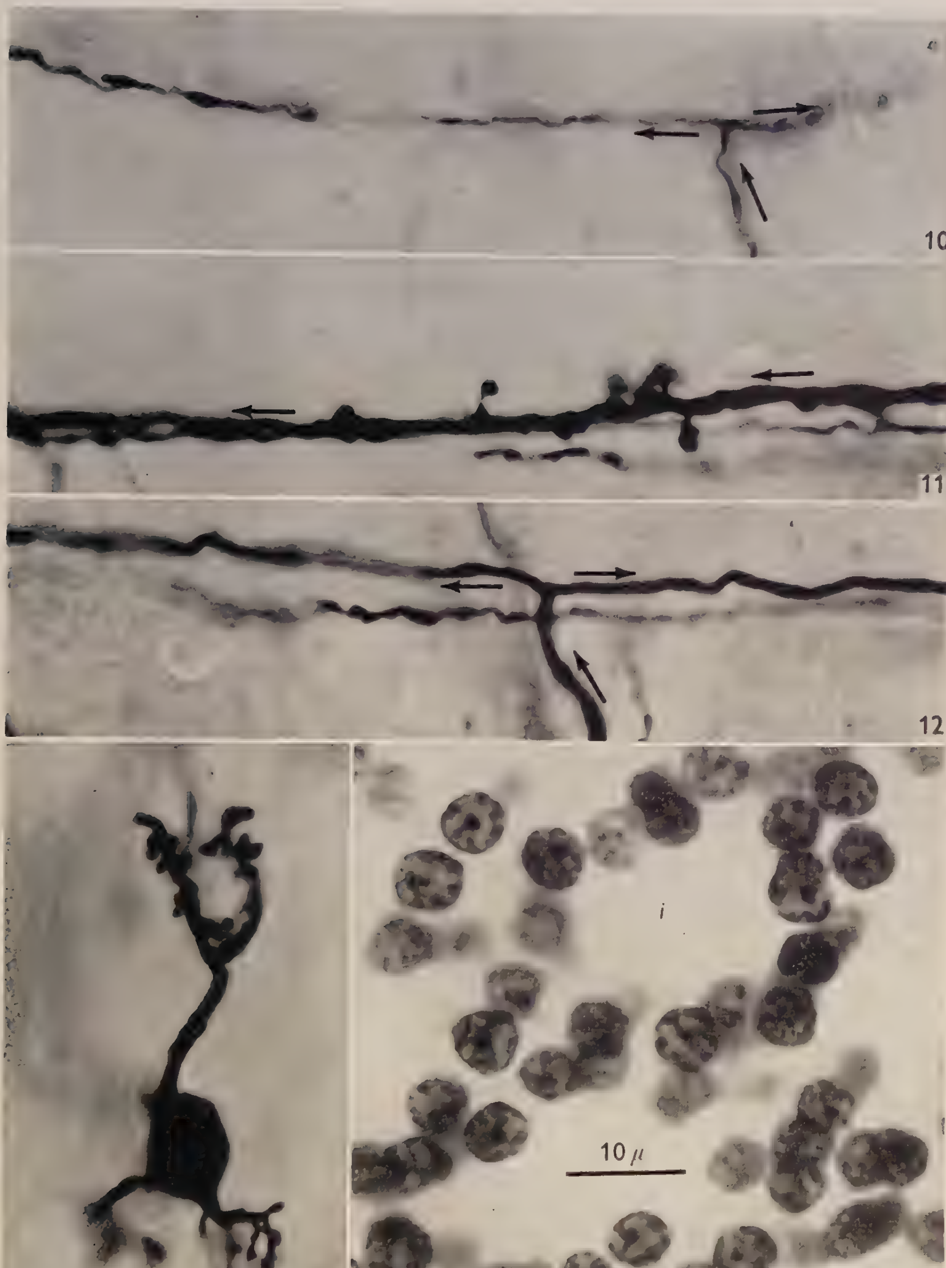

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LIST OF ABBREVIATIONS

g granular layer.
i cerebellar island.
m molecular layer.
s space for stellate cell.

EXPLANATION OF PLATES

The photomicrographs on Pl. 1 were taken under medium power and reproduced at the same magnification. All photomicrographs on Pls. 2 and 3 were taken under oil immersion and reproduced at the same magnification, except Pl. 2, fig. 7, which was taken under high power.

PLATE 1

Fig. 1. The Purkinje cell in which the total length of the spiny branchlets was calculated. Golgi preparation.

Fig. 2. A dense impregnation of parallel fibres running longitudinally in the folia. Impregnation is incomplete in the upper portion of the molecular layer. Some concept of the convergence on a Purkinje cell's branchlets may be had by imagining this stream of fibres turned at right angles to the dendritic system of the cell in fig. 1. Golgi preparation.

PLATE 2

Fig. 3. A branch of an apical dendrite of a small cortical pyramid, showing spines. Golgi preparation.

Fig. 4. The dendritic shaft of the same cell as in fig. 3, showing spines. Golgi preparation.

Fig. 5. Dendritic spines on a cell of the caudate nucleus. Golgi preparation.

Fig. 6. The spiny branchlets of a Purkinje cell. Golgi preparation.

Fig. 7. Crushed spiny branchlets of a Purkinje cell. The knob-ends of the spines, dispersed like gunshot, are rather uniform in size. Golgi preparation. High power.

Fig. 8. A portion of the dendritic system of a Purkinje cell showing the sharp contrast between the smooth branches and the spiny branchlets. Golgi preparation.

Fig. 9. A parallel fibre in a section parallel to the surface of the molecular layer. The hook-like endings are larger than the Purkinje cell's dendritic spines. Golgi preparation.

PLATE 3

Fig. 10. A superior parallel fibre, approximately $0{\cdot}2\mu$ in diameter, showing its T-shaped bifurcation. Its warty rosette endings are larger than the spines on a Purkinje cell's branchlets. Would these endings appear hook-like, if they were cut in the same plane as the fibre in fig. 9? Golgi preparation.

Fig. 11. An inferior parallel fibre showing sessile boutons, which are much larger than the Purkinje cell spines. This fibre is the direct continuation of the fibre in Pl. 3, fig. 12. Golgi preparation.

Fig. 12. An inferior parallel fibre approximately 1μ in diameter. Notice there are no endings near the T-shaped bifurcation. The first ending on this fibre is shown in Pl. 3, fig. 11, 90μ from the bifurcation. Golgi preparation.

Fig. 13. A granule cell with one dendrite and its digitiform endings in focus. The small protoplasmic processes on the digits are comparable in size to the spines on the dendrites of cells in the caudate nucleus (fig. 5). In the background unimpregnated granule cell nuclei are visible. Golgi preparation.

Fig. 14. A thin cresyl-violet section showing granule cell nuclei clustering around cerebellar islands. There is no recognizable Nissl substance in these cells.

THE SPINAL PROJECTION FROM THE NUCLEUS OF THE SOLITARY TRACT. AN EXPERIMENTAL STUDY IN THE CAT

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INTRODUCTION

It is well known that numerous visceral reflexes in which the afferent link of the reflex arc is formed by fibres in the vagal and glossopharyngeal nerves involve efferent 'centres' situated in the spinal cord. Practically all afferent glossopharyngeal and vagal nerve fibres terminate in the nucleus of the solitary tract (Torvik, 1957; and others). The impulses must, therefore, be conducted from this nucleus to the spinal cord, either through direct solitario-spinal fibres or through relay stations, e.g. in the reticular formation, that is via reticulo-spinal connexions. However, little attention has been paid to the pathways along which such impulses are conducted from the medulla to the spinal cord.

Kosaka & Yagita (1905), in Marchi material of dogs following lesions of the nucleus of the solitary tract, described direct fibres to the spinal cord. Degenerated fibres were observed chiefly in the medial part of the contralateral ventral column. These results were confirmed by Hirose (1916) in Marchi investigations on rabbits. It is, however, practically impossible to obtain lesions of the nucleus of the solitary tract without damaging adjacent fibre systems, thereby complicating the interpretation of the findings. Being aware of this, Allen (1927), using the Marchi method in the guinea-pig, concluded that no medullated fibres could be traced from the nucleus of the solitary tract to the spinal cord. Following lesions of the spinal cord at the transition to the medulla he observed slight and indefinite chromatolysis in some of the cells of the nucleus of the solitary tract. However, these changes were interpreted as being due to 'exhaustion' of the cells, caused by the large lesion, and not as true retrograde changes.

These divergent observations thus leave the question of direct solitario-spinal fibres unsolved. On account of the difficulties met with when the Marchi method is employed, it was deemed of interest to reinvestigate this problem by means of the method of retrograde degeneration. Since the acute retrograde changes usually are more clear-cut in young animals than in adult ones (Brodal, 1939; and others), the modified Gudden method (Brodal, 1940) was employed in this study. As will be described below, conclusive findings were made concerning the existence and course of the solitario-spinal fibres.

MATERIAL AND METHODS

Altogether twenty-six kittens were used as experimental animals. The animals were operated on when 6–21 days old under pentobarbital (Nembutal) anaesthesia. Following laminectomy partial lesions were made of the spinal cord at various levels by means of a

pair of sharp scissors. Penicillin was given postoperatively. None of the cases were complicated by infection.

The animals were killed by exsanguination under chloroform anaesthesia 6–12 days after the operation. The brain and part of the spinal cord were immediately dissected free and immersed in 96 % alcohol. After fixation the brain stem and the part of the spinal cord containing the lesion were embedded in paraffin and cut in 15 μ thick sections. Every 5th section was mounted and stained with thionin. The sections were then searched for the occurrence of retrograde cellular alterations in the nucleus of the solitary tract.

The brains of several animals subjected to partial cerebellar lesions, but otherwise treated in the same manner, served as controls. The cells of the nucleus of the solitary tract in this series are entirely normal.

Of the twenty-six cats operated on eleven had to be discarded because the cellular alterations were not sufficiently clear-cut, or because the lesion was inappropriate.* The findings to be presented, therefore, are based on the remaining fifteen cases.

RESULTS

Normal anatomy of the nucleus of the solitary tract in the cat

Since the solitario-spinal fibres to be described below take their origin from certain subdivisions of the nucleus only, some remarks on the normal cytoarchitecture of the nucleus are appropriate before the experimental data are described.

In thionin-stained serial transverse sections from the medulla of kittens the nucleus of the solitary tract is seen to extend from the level of the caudal pole of the facial nucleus rostrally to the rostral end of the spinal cord. Caudally the nuclei of the two sides join to form the nucleus commissuralis of Cajal (1909). The outlines of the nucleus at different levels are shown diagrammatically in Text-fig. 1.

Several authors have pointed out that in mammals the nucleus of the solitary tract is composed of structurally different subdivisions (Cajal, 1909; Allen, 1923*a*; Meessen & Olszewski, 1949; Olszewski & Baxter, 1954; Torvik, 1957; Brodal, Szabo & Torvik, 1957). Particularly clear-cut are the differences between the medial and lateral parts of the nucleus (Cajal; Torvik; Brodal, Szabo & Torvik). In kittens these mediolateral differences are very distinct at certain levels (Text-fig. 1; Pl. 1, fig. 1, *m.*, *l.*). The medial division (*m.*) is situated dorsal and dorsolateral to the motor vagal nucleus, and appears as a structurally uniform column throughout the longitudinal extent of the nucleus. It is composed of densely packed small pale nerve cells and closely resembles the substantia gelatinosa of the dorsal horn in the spinal cord. The lateral division (*l.*), which is not clearly delimited from the medial part, is found as a looser column with larger cells surrounding the fibres of the solitary tract. It is easily recognized in the caudal three-quarters of the nucleus, but becomes indistinct above the rostral pole of the hypoglossal nucleus, where the medial and lateral parts join, and practically all cells are of the small variety. Three types of cells can be recognized in the lateral division: large, medium-sized and small ones. The large cells are found only in the middle half of the rostrocaudal extent of the nucleus. They are rather scanty and situated chiefly in a semicircle ventral to the

* It is well known that in young animals the survival period which is necessary for the development of retrograde cellular changes varies, even in animals with the same age and weight. This variation is probably due to individual differences in the speed of development of the central nervous system.

solitary tract (Pl. 1, fig. 3). From one to ten large cells are found on each side in a section. They are multipolar and somewhat larger than the cells in the dorsal motor vagus nucleus. The Nissl substance often tends to accumulate along the cell membrane, and the nucleus is frequently excentric. The medium-sized cells (Pl. 1, fig. 3) which are rather numerous, have approximately the same size as those in the dorsal motor vagus nucleus. They are found along the whole rostro-caudal extent of the lateral division, but are somewhat more scanty towards the caudal end. Most of these cells are found in the ventral part of the lateral division, but scattered medium-sized cells occur also in the dorsal part. Most of them are multipolar and somewhat elongated, with rather evenly distributed Nissl substance. The small cells are of the same type as those in the medial division of the nucleus.

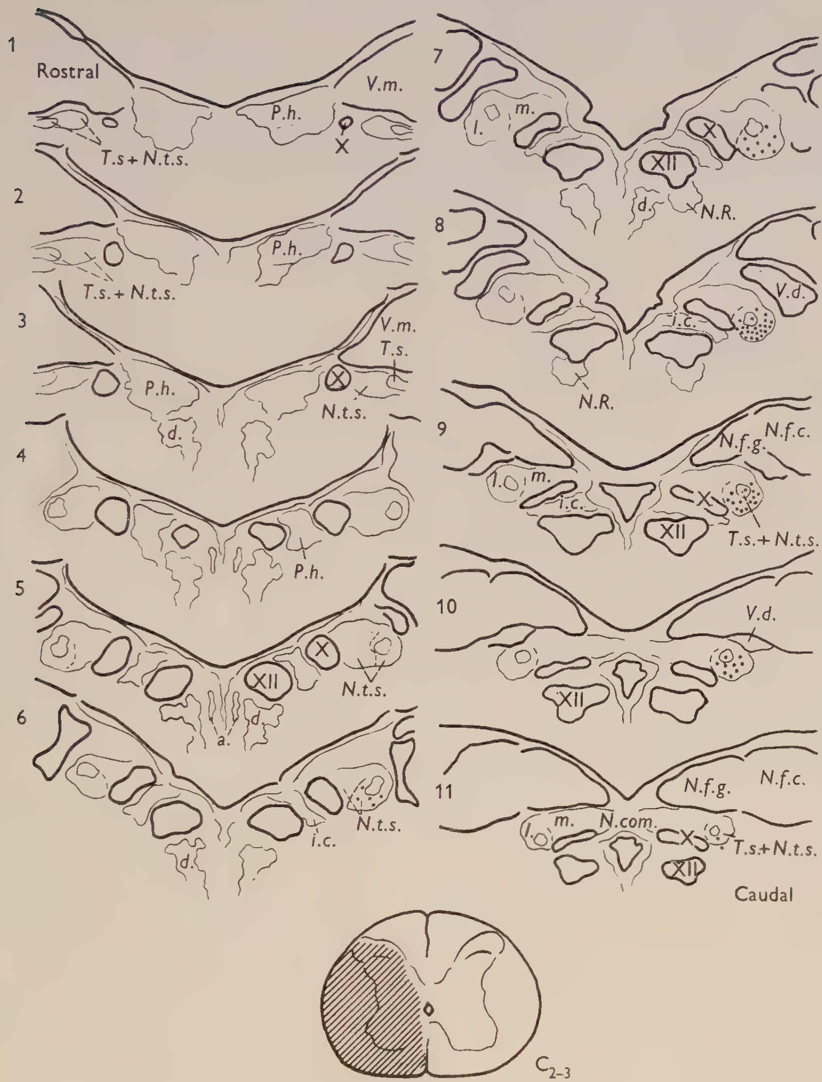
Retrograde cellular changes in the nucleus of the solitary tract following lesions of the spinal cord

Following appropriately placed lesions of the spinal cord (see below) characteristic retrograde cellular changes are found in the lateral division of the nucleus of the solitary tract. Examination of cases with different survival periods after the operation shows that the typical nerve cell changes appear 7–8 days after the lesion. At this time the affected regions of the nucleus also show a definite loss of nerve cells. The acute retrograde changes in the nerve cells of the nucleus are characterized by rounding of the cell contour, extreme chromatolysis, and peripheral displacement of the nucleus (Pl. 1, figs. 4, 5). The chromatolysis is particularly characteristic and leaves the cytoplasm quite homogeneous with a bluish, 'milky' appearance. The nuclear membrane is often folded, and the nucleolus frequently somewhat displaced. Convincing changes have been observed only in the large and medium sized cells in the lateral division of the nucleus, while the small cells appear to be unchanged. The loss of nerve cells is also confined to the large and medium-sized cells. Only areas showing unequivocal retrograde nerve cell changes of the type described, or definite nerve cell loss, have been taken into account in this study.

Site of origin and course of the solitario-spinal fibres

In order to determine the maximal changes in the nucleus of the solitary tract following spinal cord lesions a series of cases with more or less complete hemisections of the cord at the level of C_{2-3} were examined. The following experiment is representative.

Cat D49. Operated on 8 days old, killed after 8 days. (Text-fig. 1 and Pl. 1, figs. 1–5.) Transverse serial sections through the rostral part of the spinal cord show that the lesion is strictly unilateral. The dorsal funiculus and a very small part of the ventral funiculus is spared on the side of the lesion (Text-fig. 1). The nucleus of the solitary tract exhibits acute retrograde cellular changes and heavy nerve cell loss in certain regions. The changes are confined to the lateral division of the contralateral nucleus. The relative intensity at different levels and the distribution of the changes are shown diagrammatically in Text-fig. 1. The heaviest changes are found in the middle third of the rostrocaudal extent of the nucleus, where most of the large cells are situated. Towards the rostral and caudal ends of the lateral division the changes are more sparse. Practically all large cells and between one-half and three-fourths of the medium-sized ones in the contralateral nucleus have



Text-fig. 1. Diagram summarizing the findings in cat D49. In a series of drawings taken at equal intervals from a series of transverse Nissl-stained sections through the lower part of the medulla, dots indicate nerve cells showing retrograde changes or areas with definite nerve cell loss in the nucleus of the solitary tract. Below a drawing showing the extent of the lesion at C_{2-3}.

Abbreviations employed in Text-fig. 1, *and Pl.* 1, *figs.* 1 *and* 2:

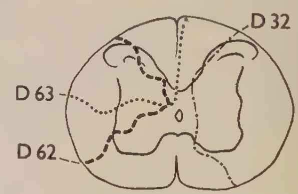
a.	Paramedian reticular nucleus of Brodal, accessory group	*N.f.g.*	Nucleus funiculi gracilis
d.	Paramedian reticular nucleus of Brodal, dorsal group	*N.R.*	Nucleus of Roller
i.c.	Nucleus intercalatus of Staderini	*N.t.s.*	Nucleus of the solitary tract
l., *m.*	Lateral and medial division, respectively, of nucleus of the solitary tract	*P.h.*	Nucleus praepositus hypoglossi
N.com.	Nucleus commissuralis of Cajal	*T.s.*	Solitary tract
N.f.c.	Nucleus funiculi cuneatus	*V.d.*	Descending vestibular nucleus
		V.m.	Medial vestibular nucleus
		X.	Dorsal motor vagal nucleus
		XII.	Hypoglossal nucleus

disappeared or show clear-cut retrograde changes (Pl. 1, figs. 1–5). No convincing acute retrograde changes or definite reduction in number have been observed in the small cells of the nucleus.

An identical distribution of cellular changes was found in the nucleus of the solitary tract in cat D 19 (complete hemisection of the cord at the level of C_{2-3}) and in cat D 44 (almost complete hemisection at the same level). Both animals were operated on 8 days old, and killed 8 days later.

The consistent findings in these cases, as well as in others to be dealt with below, permit the conclusion that the solitario-spinal fibres take origin from the lateral division of the solitary tract. All descending fibres from the nucleus cross the midline above the second cervical segment. Practically all large cells and between one-half and three-fourths of the medium-sized ones project to the spinal cord.

Information of the localization within the spinal cord of the solitario-spinal fibres was obtained from cases with more restricted lesions at the level of C_{2-3}. In two animals with lesions restricted practically to the lateral funiculus (and part of the grey substance) of the cord (cat D 32, operated on 21 days old, killed 9½ days later; cat D 62, operated on 8 days old, killed 7½ days later, Text-fig. 2), the changes in the nucleus of the solitary tract are of practically the same intensity as in cat D 49 (Text-fig. 1; Pl. 1, figs. 1, 2). Corresponding observations have been made in several other cases where the ventral funiculus is spared.



Text-fig. 2. Drawing showing the extent of the lesions in cats D 32, D 62 and D 63 (level of C_{2-3}).

Following an isolated lesion of the dorsal funiculus (cat D 33, operated on 21 days old, killed after 8 days), no changes are found in the nucleus of the solitary tract. Other cases with lesions involving the dorsal funiculi in addition to the ventrolateral funiculus also show that the lesion of the dorsal funiculi does not produce changes in the nucleus of the solitary tract. Thus in cat D 49 (Text-fig. 1), the changes in the nucleus are even slightly more pronounced than in cat D 19, where there is a complete hemisection of the spinal cord.

In cat D 63 (operated on 8 days old, killed 8 days later) a unilateral lesion at the level of C_{2-3} spares the ventral half of the lateral funiculus (Text-fig. 2). Retrograde changes are found in the same regions of the nucleus of the solitary tract as in the cases described above. However, the changes are less marked than in the cases with larger lesions.

Although no cases with lesions restricted to the ventral funiculus have been studied, it may be concluded from the available material that most, if not all, of the solitario-spinal fibres descend in the lateral funiculus of the spinal cord. The findings in cat D 63 indicate that the fibres are located in the dorsal as well as in the ventral half of the lateral funiculus.

Several experiments have been performed to decide how far caudalward the solitario-spinal fibres descend. Cases with lesions at the level of Th_1 demonstrate that practically all fibres descend beyond this level. Thus in cat D 65 (operated on 9 days old, killed 8 days later) a complete hemisection at the level of Th_1 resulted in heavy retrograde cellular changes in the opposite nucleus of the solitary tract. The changes are located to the same region and are of approximately the same intensity as in

cat D49 (see above and Text-fig. 1). Similar, though slightly less intense, changes were found in another case with an approximate hemisection at the same level (cat D53, operated on 8 days old, killed 8 days later).

In six animals with extensive lesions at the level of L_1 (cats D36, 37, 45, 46, 66, 67) no changes could be detected in the nucleus of the solitary tract. Although negative results cannot be considered decisive, the consistent findings in all these cases strongly support the assumption that the number of solitario-spinal fibres possibly descending below the level of L_1 must be very modest.

DISCUSSION

The acute retrograde cellular changes found in the nucleus of the solitary tract following lesions of the spinal cord are characteristic and closely resemble the retrograde changes described in several other nuclei in the brain stem of kittens (see, for example, Brodal, 1940; Brodal & Jansen, 1946; Torvik & Brodal, 1957). The identical distribution of these changes in cases with the same type of lesion leaves no doubt that the cellular alterations observed and the later occurring cell loss in the nucleus of the solitary tract are due to transection of the axons of the nerve cells in question.

Only clear-cut cellular changes or a definite loss of nerve cells have been considered positive in the present study. Definite changes have not been observed in the small cells of the nucleus. However, since the acute retrograde changes in small cells are less clear-cut than in larger ones, it cannot be excluded that some of them may project caudally. Furthermore, it appears that not all types of nerve cells do react with definite retrograde changes or disintegration following transection of their axons. It is of interest in this connexion that in a rather large series of kittens subjected to hemisection of the brain stem at mesencephalic or thalamic levels no convincing changes could be detected in the nucleus of the solitary tract 4–13 days after the making of the lesion. It is possible, therefore, that the number of caudally projecting neurons in the nucleus of the solitary tract may virtually be larger than appears from the findings made in this study, and that also some small cells may project to the cord.

The solitario-spinal fibres observed in this study take their origin from the lateral magnocellular part of the nucleus and descend in the opposite lateral funiculus of the spinal cord. No evidence was obtained that fibres descend in the ventral funiculus, as described by Kosaka & Yagita (1905) and Hirose (1916). Probably the degenerated fibres described by these authors belong to other fibre systems which have been inadvertently damaged, e.g. reticulo-spinal fibres.

Cajal (1909), in Golgi material of the mouse, observed axons from the cells in the lateral division of the nucleus of the solitary tract ('ganglion descendent') which dichotomize, sending one branch rostrally and the other to the spinal cord. The absence of changes in the nucleus of the solitary tract following rostral lesions does not permit any conclusion as to whether the caudally projecting neurons identified here also have ascending axonal branches. However, according to Allen (1923*b*) the neurons which project in a rostral direction are located in the rostral half of the nucleus only, while the changes observed in this study are confined chiefly to the caudal part of the nucleus.

Some information of the function of the solitario-spinal fibres may be obtained from a consideration of the afferent connexions of the neurons which project caudally. Experimental investigations on the afferent connexions of the nucleus of the solitary tract in mammals (van Gehuchten, 1900; Allen, 1923*a*; Du Bois, 1929; Torvik, 1957; and others) show that the primary sensory nerve fibres to the nucleus terminate at different levels in the nucleus, the terminal region of the intermediate (facial), glossopharyngeal and vagus nerves following each other rostrocaudally in this order. In an experimental study in the rat (Torvik, 1957) a considerable contingent of trigeminal fibres was also traced to the nucleus of the solitary tract (method of terminal degeneration). These fibres terminate at the level of entrance of the glossopharyngeal nerve fibres. Since the rostral limit of the changes in the present study lies considerably caudal to the entrance of the afferent glossopharyngeal nerve fibres, the changed areas of the nucleus are probably supplied with primary afferent fibres chiefly from the vagal and glossopharyngeal nerves. One of the functions of the solitario-spinal fibres, therefore, is probably to conduct to the spinal cord reflex impulses entering via these nerves. It is in accord with this suggestion that most of the solitario-spinal fibres terminate in the thoracic spinal cord, i.e. at levels where the intermediolateral cell column and the motor neurons to the respiratory muscles are situated. (Whether the fibres give off collaterals to the cervical segments cannot be decided from the present material.)

Present knowledge shows that the nucleus of the solitary tract cannot be considered as being only a relay nucleus in the central pathways for primary afferent nerve impulses. It also appears to serve as an integrative centre. The experimental demonstration that, in the cat and rat, fibres arising in different areas of the cerebral cortex terminate in all parts of the nucleus of the solitary tract (Brodal, Szabo & Torvik, 1957; Torvik, 1957) indicates that the cortex may influence the central transmission of visceral impulses through the nucleus. Furthermore, it has been found (in the rat and cat) that a small number of ascending fibres from the spinal cord terminate in the nucleus (Torvik, 1957; Rossi & Brodal, 1957). Thus there appear to be possibilities for an interaction of spinal, cortical and primary sensory impulses within the nucleus. It is of interest in this connexion that in an experimental study on the reticulospinal projection in the cat (Torvik & Brodal, 1957) no descending fibres could be detected from the dorsolateral part of the reticular formation of the medulla, which is said to harbour several 'centres' which transmit their impulses to the spinal cord (expiratory centre, Pitts, Magoun & Ranson, 1939; vasomotor centres, Alexander, 1946; vomiting centre, Wang & Borison, 1950; and others). Exact knowledge of the connexions from the reticular formation to the nucleus of the solitary tract is lacking. However, if such connexions exist, the solitario-spinal fibres might also be concerned with the transmission of impulses from these 'centres' to the spinal cord.

SUMMARY

The descending projection from the nucleus of the solitary tract to the spinal cord has been investigated in young kittens by means of the modified Gudden method of Brodal (1940).

Following approximate hemisections of the spinal cord at the level of C_{2-3} clear-cut acute retrograde cellular changes and definite loss of nerve cells are found in the lateral magnocellular division of the contralateral nucleus of the solitary tract (see Text-fig. 1 and Pl. 1, figs. 2, 4 and 5). The changes are confined to the large and medium-sized cells.

Cases with lesions restricted to parts of the transverse sectional area of the cord show that the solitario-spinal fibres descend in the lateral funiculus of the cord. Most of the fibres terminate in the thoracic cord, while very few, if any, descend below the level of the first lumbar segment.

The localization of the changes in the nucleus of the solitary tract indicates that the primary afferent fibres to the neurons projecting caudally are derived from the vagal and glossopharyngeal nerves. The functional implications of the findings are briefly discussed

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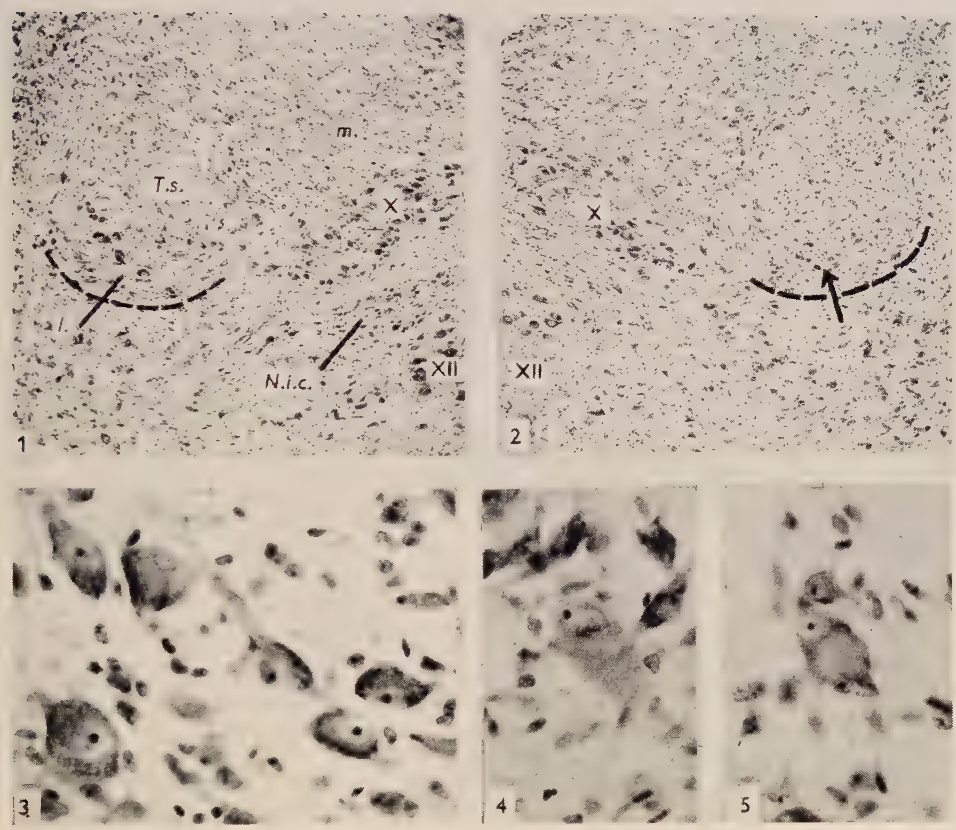
EXPLANATION OF PLATE

Figs. 1, 2. Photomicrographs of a transverse section through the nucleus of the solitary tract in cat D 49. Fig. 1. The normal nucleus on the side of the lesion. Fig. 2. Nerve cell loss (arrow) in the lateral division of the nucleus on the side opposite to the lesion. $\times 50$.

Fig. 3. Photomicrograph of normal large and medium-sized cells in the lateral division of the nucleus of the solitary tract. $\times 350$.

Figs. 4, 5. Photomicrographs of two cells (large and medium-sized) showing retrograde cellular changes in the lateral division of the nucleus of the solitary tract. Cat D 49. $\times 350$.



TORVIK—SOLITARIO-SPINAL FIBRES

THE DEVELOPMENT OF THE PRIMARY SENSORY SYSTEM IN *XENOPUS LAEVIS* (DAUDIN)

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Anatomy School, University of Cambridge

INTRODUCTION

The development of the nervous system is usually studied with particular ends in view. Many authors have been concerned with functional development, with the correlation of emerging patterns of behaviour and structural organization (Coghill, 1929; Hooker, 1952; Whiting, 1955). For others, neuroembryology is a branch of developmental mechanics (Detwiler, 1936; Piatt, 1948; Hamburger, 1952). A third group, by far the smallest at present, is concerned primarily with the physiology of the developing nerve cell (Flexner, 1955; Hughes, 1955). The degree to which these groups of researches have so far been distinct is perhaps an indication of how much yet remains to be learnt about the developing nervous system. Mature and comprehensive treatment of the subject will ultimately demand the correlation of all these approaches.

In an attempt to investigate aspects of the subject which belong to the second and third of these divisions, the toad *Xenopus laevis* has been selected for study among Amphibia, in the first place mainly because of the great convenience that fertile eggs may be obtained at any time of year.

Examination of stages in the development of the nervous system of this species revealed features of interest in its development, which may lend themselves to further investigation by experimental and histochemical methods. The present study describes some aspects of the development of the sensory system of the trunk. The embryology of the nervous system of *Xenopus* apparently has not hitherto been studied, apart from general accounts of the development of this species, such as those of Weisz (1945 *a–c*). In these papers, however, the author hardly does justice to the pioneer work of Bles (1905) on the development of this amphibian.

Recently, Nieuwkoop & Faber (1956) have published an extremely valuable work on its development, which will long serve as the basis of all future studies on *Xenopus*. The present observations, however, were completed before this work became available.

MATERIAL AND METHODS

Embryos and larvae were obtained from the colony which has been founded by my colleague Dr Pierre Tschumi, to whom I am deeply grateful for his generous and unfailing collaboration. Dr Tschumi used chorionic gonadotropin (Ciba, Basle) to induce both amplexus and ovulation (Gasche, 1943; Ochsé, 1948).

The material was studied by several methods after sectioning. For the general cellular arrangement of the cord, staining by routine methods such as Ehrlich's haematoxylin and eosin was adequate. Such preliminary study was followed by the preparation of section series stained with silver by methods of impregnating sections

already mounted on the slide. At first the technique of Holmes (1947) was used, which has been found to give good results with both chick and fish material. In early stages of the development of *Xenopus* adequate results are often obtained with Holmes's method, which, however, proved unreliable with later material. Fortunately, it became possible to apply the Bodian technique which uses a silver proteinate, thanks to an available supply of a suitable form of this substance from Établissements Roques (Paris). With *Xenopus*, only the best Holmes preparations are comparable with those obtained by Bodian's method.

In the first phases of their growth, nerve fibres have only a feeble affinity for silver, so much so that in the past, as in Coghill's researches (e.g. 1914), the developing amphibian nervous system has been studied in sections stained only with dyes. It has been found, however, that the phase-contrast principle is of great help in studying these early stages after silver impregnation. Under the phase microscope contrast and sharpness in such preparations are greatly enhanced.

The developing spinal cord of *Xenopus* has also been studied by means of the ultraviolet microscope. It is intended to make a comparative survey of the distribution of nucleic acids within the cord of a series of vertebrate embryos, and a study of that of the chick has already been published (Hughes, 1955). The technique described in that paper has been used for the present work, that is to say unstained sections cut at $2{\cdot}7\,\mu$ in ester wax are photographed at 2537 A. Here, however, a glycerin-immersion monochromat of N.A. 1·25 has been used throughout. The cord of *Xenopus* during the earlier part of larval life is sufficiently small for it to be possible to cover its whole area in several contiguous fields with this objective. The resulting photomicrographs at $\times$ 2000 are combined into a complete transverse section of the whole cord.

For work in the ultraviolet the fixative devised by Serra (1946) has been employed, largely for uniformity with the previous study on the chick. This mixture was found to give a satisfactory fixation for *Xenopus* material. It was further found that embryos and early larvae fixed by this means could be used for the Holmes method of silver impregnation, where fixatives such as Bouin's fluid are unsuitable. When larvae are more than 10 mm. in length, it is desirable to sever the tail at fixation, both to facilitate the penetration of the fixative and also to prevent distortion of the notochord. In the later stages of larval life the notochord is very large and approaches 1·0 mm. in diameter. If a fixative is allowed to penetrate slowly through the notochordal sheath, the whole structure collapses, with consequent grave distortion of the surrounding tissues.

EXTRA-GANGLIONIC SENSORY NEURONES

(1) *Historical*

In the lower vertebrates it is well known that there are sensory neurones at trunk levels which do not belong to the dorsal root ganglia. There are apparently more than one series of these elements. One set is now generally known as the Rohon-Beard cells, after Rohon (1885) and Beard (1889, 1892, 1896), who have described them in several groups of fishes. These cells have also been called after other investigators, namely Reissner (1860) and Sigmund Freud (1877) both of whom

studied them in *Petromyzon*. The latter referred to them as 'Hinterzellen'. In *Petromyzon*, these cells persist into adult life, but they are found only in embryonic or larval stages in Elasmobranchs (Beard, 1892, 1896; Neal, 1914), in most Teleosts (Harrison, 1901) and in the Amphibia (Burckhardt, 1889; Studnička, 1896; Coghill, 1914).

In a large proportion of the Teleostei, which Dahlgren (1898) estimates to be over half the total number, there is a further system of supra-medullary sensory neurones. They are found either dorsal to the cord, as in *Lophius* (Fritsch, 1886), or within the dorsal fissure, e.g. in *Ctenolabrus* (Sargent, 1899), and in the Heterosomata (Dahlgren 1898). In *Lophius* they are found in both positions (Kappers, Huber & Crosby, 1936, I, fig. 81). These cells may be of great size. In *Lophius*, Fritsch's drawing suggests that they may be over 200μ in diameter; they are penetrated by capillaries, and also by the canals of Holmgren (Holmgren, 1899). These neurones are generally multipolar in form; the course of both their afferent and efferent fibres is variable from species to species, and diverse functions have been assigned to them. In the sunfish *Orthagoriscus*, on the basis of their rich blood supply, Burr (1928) has suggested that they may be chemo-receptors. Whiting (1956 *b*) suggests that the term 'Fritsch cell' is appropriate for neurones of this type.

On these neurones much has been written; in 1899 Sargent counted over sixty papers in this field. Yet only for the Rohon-Beard cells has any systematic study been made of their development. Each type may well be derived from the neural crest, though it is only for the Rohon-Beard cells of *Amblystoma* that any experimental evidence is available (DuShane, 1938). It is not clear how far supra-medullary neurones are homologous with Rohon-Beard cells, nor is it certain that all those to which this name has been given are necessarily of the same category.

Where sufficient information is available it seems that in Gnathostomes, the Rohon-Beard cells develop first in dorsolateral positions, from which they later move towards the mid-line. Most of the evidence for this point relates to the Amphibia, and will be discussed in the succeeding section. Among the Teleostei, Harrison (1901) described how in the development of the salmon, the 'Hinterzellen' migrate medially, and in so doing change from a bipolar to a monopolar form. At later stages, when the yolk-sac disappears, these cells degenerate. Rohon (1885), in his paper on the trout, describes bipolar cells in a dorsal position at stages soon after hatching; these are also shown in van Gehuchten's figures (1895) of similar stages; Rohon, however, in an 'erwachsenen grösseren Forelle' shows large multipolar cells between white matter and the dorsal horn; but whether these are Rohon-Beard or Fritsch cells is not clear.

Instances are known where extra-ganglionic sensory neurones at early stages are found near to the median plane; Dahlgren (1897) describes a double row of giant ganglion cells in the larva of a flat fish (*Bothus maculatus*) which was no more than 3 mm. in length. Beard (1892) shows the 'transient ganglion cells' of *Raja batis*, at their first appearance as a group of dorsally situated cells, and claimed that some of them afterwards migrate away from the cord towards, and even into, the somites, though Harris (1956) is unable to confirm Beard's account in this respect. Harris is of the opinion that neurones of both the Rohon-Beard and of the Fritsch series are present in Elasmobranch embryos.

The Rohon-Beard cells in Amphibia

The presence of Rohon-Beard cells in Amphibia was first recognized in 1889. In that year, Burckhardt published an account of the spinal cord in *Triton* relating mainly to post-larval stages, in which these cells are then found in mid-dorsal positions, most abundantly at caudal levels. They were found to be highly variable in form. In that same year, there also appeared Beard's first paper, primarily concerned with the early development of *Lepidosteus*, in which he noted the presence of the 'transient or larval nervous apparatus'. He compared its constituent giant ganglion cells with those in other forms, noting that 'they may be found in larvae of *Rana* and *Triton*'; in the latter he followed them to their final atrophy.

Seven years later, there appeared a study by Studnička (1896) on the histogenesis of the spinal cord, which ranged from *Amphioxus* to the Amphibia. In the latter group, he was mainly concerned with the Anura. Rohon-Beard cells were recognized in *Rana*, *Bufo*, *Pelobates* and *Bombinator*. He showed that in *Bufo*, these cells were dorsolateral at the 3 mm. stage, but by 6 mm. had moved to a mid-dorsal position. In later larvae of *Bombinator*, the Rohon-Beard cells move still farther dorsalwards, and come to lie within the white matter in a median plane. At the approach of metamorphosis, they begin to degenerate.

Among the Urodeles, Studnička could not find Rohon-Beard cells in a 30 mm. *Salamandra*, but recognized them at various stages in *Triton*, in a 20 mm. larva of which they were found in a mid-dorsal position.

Studnička traced lateral processes from the Rohon-Beard cells to the septa between the myotomes, and concluded, as had Beard in the Elasmobranchs, that these neurones were motor in function.

One year later, van Gehuchten in the course of a series of comparative studies on the spinal cord, in which silver impregnation by Golgi methods was employed, concluded (1897 *a*) that not all cells in dorsal positions within the cord are homologous. He distinguished between true Rohon-Beard cells which gave off lateral branches, sometimes in the dorsal roots, and other mid-dorsal neurones whose fibres are confined to the cord, such as he had found in larval stages of *Salamandra* and outside the Ichthyopsida, in embryos of the grass-snake.

There were no further accounts of Rohon-Beard cells in Amphibia until Coghill in 1914 began his series of numbered papers on the developing nervous system of *Amblystoma*, though in 1910 Harrison had described the outgrowth of fibres from these neuroblasts in *Rana*, both normally and after explanation in tissue culture.

Coghill was interested in the emergence of behaviour patterns, outside of which he was not concerned with morphological questions. His investigations, as he said, 'were not undertaken with the idea of discovering new things in anatomy'. By the early years of this century, however, much had been learnt of the general principles on which the nervous system is arranged. In particular, the concept of the proprioceptive sense had been established by Sherrington (1906). On the basis of correlations between neuroembryology and behaviour at each stage in *Amblystoma*, Coghill in 1914 interpreted the branches of the Rohon-Beard cells between the myotomes as proprioceptive, though at this period of development these fibres are without any special sensory endings.

Coghill's description of the Rohon-Beard neurones in *Amblystoma* extends through a period which includes four phases in the development of behaviour. These are known as the 'non-motile', the 'early-flexure', the 'coil', and the 'early swimming' stages. They correspond respectively with nos. 29, 31, 34 and 36 of Harrison's series (Rugh, 1948). The Rohon-Beard cells throughout this period remain in a dorsolateral position, though this varies at different levels according to the degree to which the myotomes extend dorsally. These Rohon-Beard cells are most densely distributed caudally where, opposite each myotome, there may be up to ten on each side. There is little or no increase in this number after the non-motile stage.

There is no adequate description of the Rohon-Beard cells in *Amblystoma* after the period with which Coghill was concerned in this paper. Hutchinson (1936) illustrates them still dorsolateral in position at stage 39. One such cell is shown, however, near the mid-line in fig. 1 of DuShane's paper (1938). This is in the host cord of an experimental larva into which another had been grafted. It is not possible to judge the stage in normal development to which this larva corresponds.

Coghill showed that in *Amblystoma* the peripheral branch of a Rohon-Beard cell runs in the septum between adjacent myotomes to innervate both skin and muscle. It is thus both exteroceptive and proprioceptive in function. The central branches of Rohon-Beard cells run forwards in the dorsal zone of the cord to form a sensory column. This primary afferent system persists until it is superseded by the development of the dorsal root ganglia.

Processes of the Rohon-Beard cells can be traced both to skin and myotomes in the non-motile embryo. Within the cord, longitudinal processes from these cells are first seen at mid-trunk levels. By the time when the larva begins to swim, these processes have developed into a continuous tract of fibres throughout most of the length of the cord.

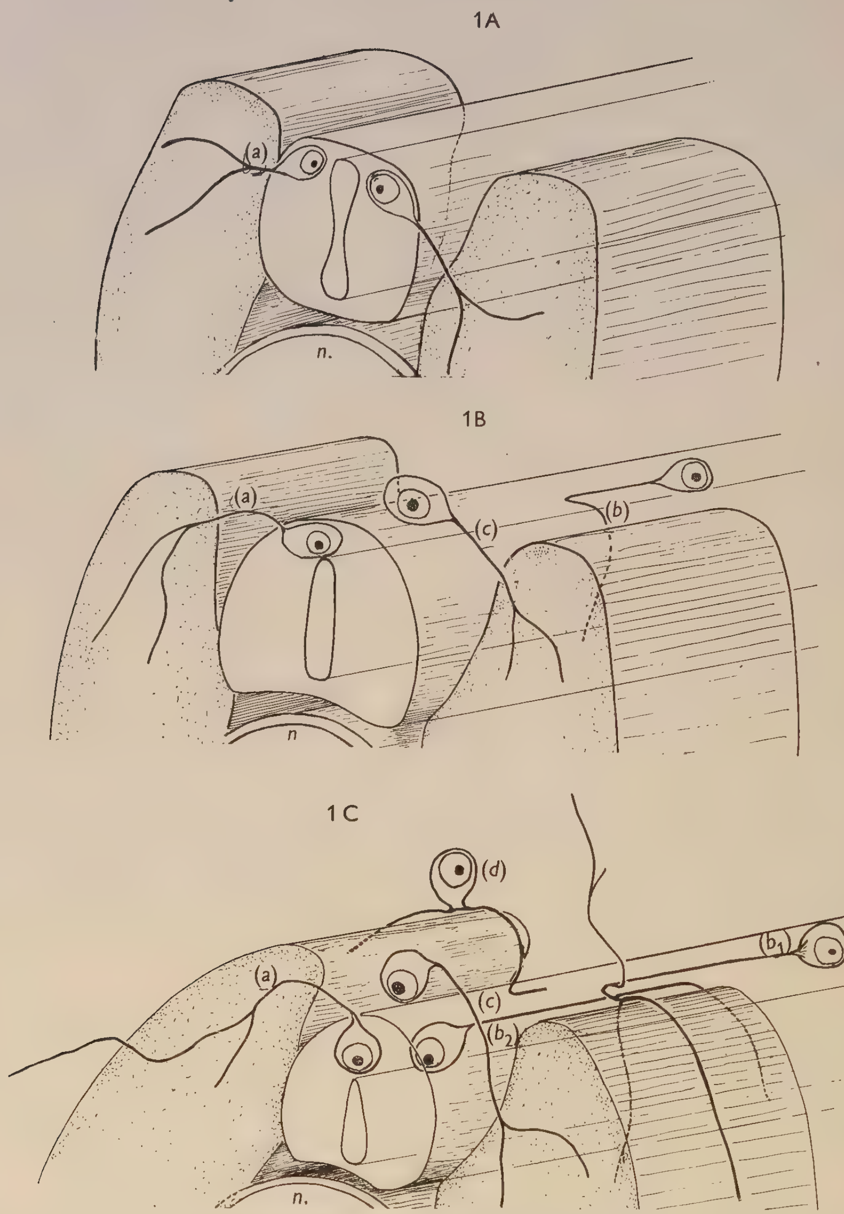
Concerning the peripheral branches of the Rohon-Beard cells in *Amblystoma*, Coghill stated (1914, p. 186) that, although 'the great majority of fibres of this system pass out of the cord into intimate relation with the ends of the myotomes, there may be in the later stages an increasing number that pass out to the skin over the middle portion of the myotomes'.

(2) *Rohon-Beard cells in* Xenopus

Nieuwkoop & Faber (1956) include among the features which characterize each stage of development the times at which Rohon-Beard cells are first visible in *Xenopus* and also those at which the cells are no longer present. Their findings generally correspond with those of the present work, which will now be described.

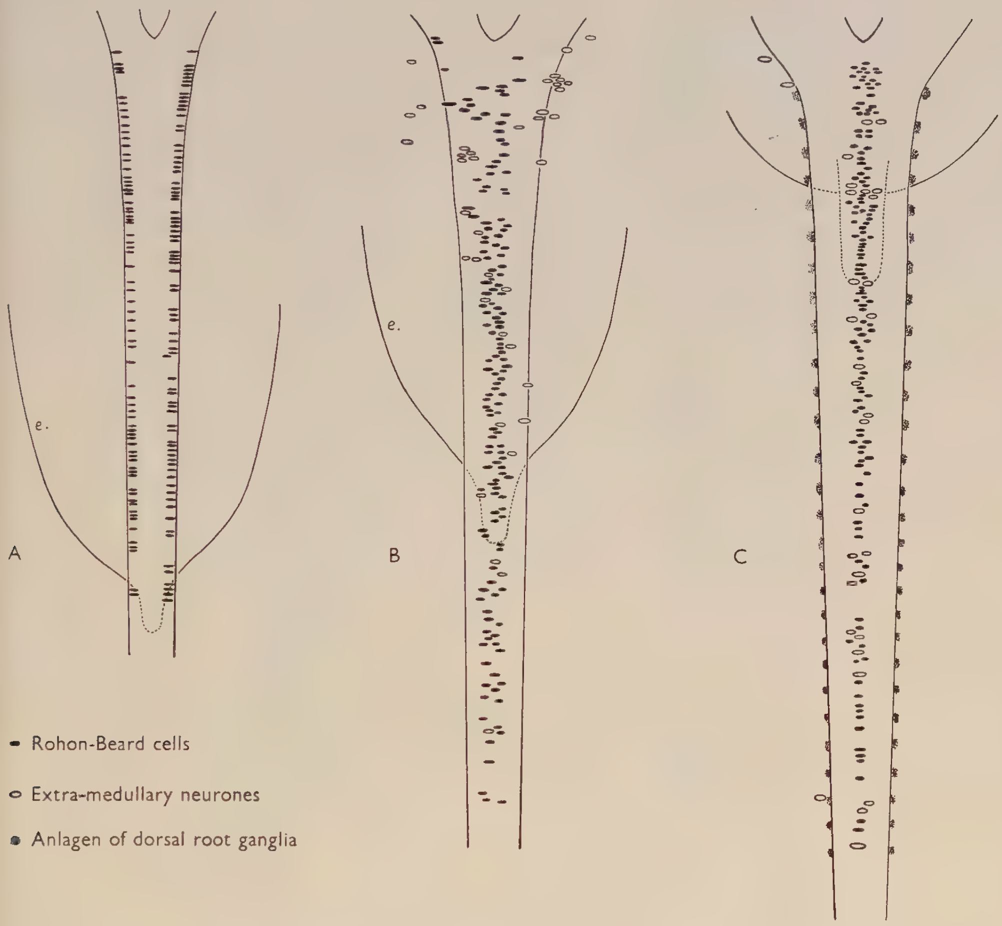
In *Xenopus*, the Rohon-Beard cells are recognizable in the cord at 43 hr., when they are ovoid and dorsolateral in position as in *Amblystoma* (Text-figs. 1 A, 2 A; Pl. 1, fig. 1). At this stage, lateral processes can be traced only from those cells which lie opposite the septa between the myotomes, where some have already become irregularly branching nerve fibres with a weak affinity for silver. Within the cord, no longitudinal processes from the Rohon-Beard cells are yet seen.

Some of the characteristic features of the first sensory system of *Xenopus* are already visible at 58 hr. (Text-fig. 1 B). Throughout the cord the Rohon-Beard cells are then moving towards the mid-line. Some have reached this position, though



Text-fig. 1. Diagrams to illustrate the development of the primary sensory system in *Xenopus*: (A) at 43 hr.; (B) at 58 hr.; (C) at 106 hr. *n.*, notochord. (A) Dorsolateral Rohon-Beard cells send dendrites (*a*) to the septa between myotomes. (B) In addition, a lateral branch (*b*) of a longitudinal Rohon-Beard fibre runs down on the inner surface of a myotome. The dendrite of an extra-medullary neurone (*c*) has the same course as (*a*). (C) Some dendrites of (*a*) now run forward underneath the ectoderm. Branches of (*b*) ascend to the median fin. Lateral branches from the longitudinal Rohon-Beard tract are given off by both ascending (b_1) and descending (b_2) fibres.

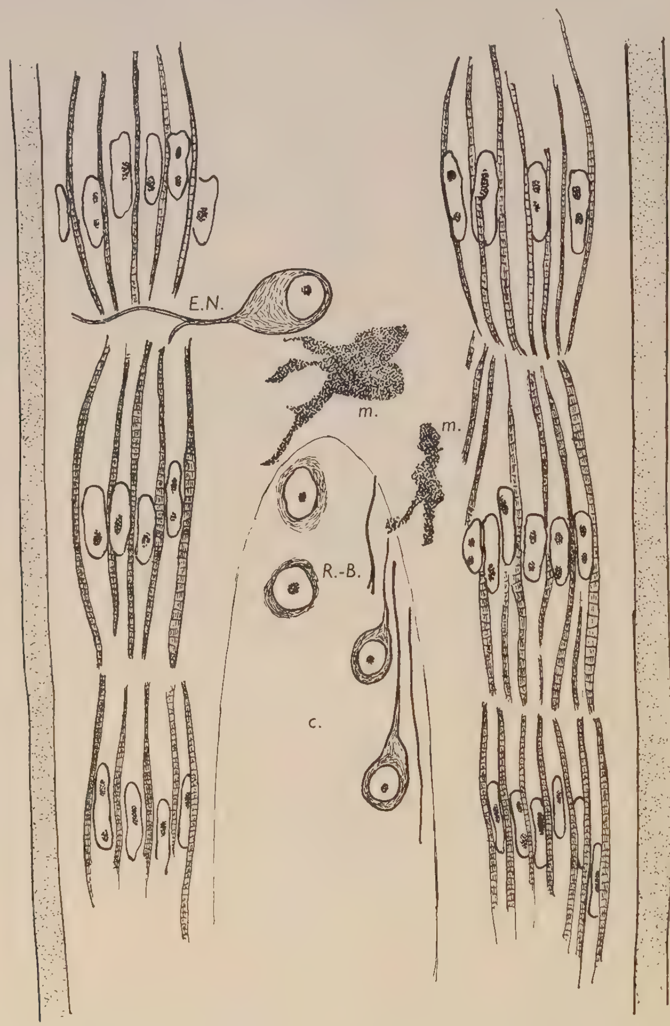
where two Rohon-Beard cells from opposite sides meet they are often mutually impeded in their final stages of migration. Sections at a small angle to the horizontal plane have been found of great value in tracing the course of the fibres from the Rohon-Beard cells. Text-figs. 3 and 4 are drawings of two such sections from series prepared by Holmes's method which cut the dorsal surface of the cord at an acute angle.



Text-fig. 2. Diagrammatic reconstruction of the dorsal aspect of the spinal cord in *Xenopus* to show the distribution of Rohon-Beard cells within the cord and of extra-medullary neuroblasts: (A) at 43 hr.; (B) at 81 hr.; (C) at 154 hr. In (A) and (B) the caudal margin of the yolky endoderm is shown (*e.*) beneath the cord. In (C) the position of the rectum and of the hinder limits of the body cavity are similarly shown. In (C) the anlagen of the dorsal root ganglia are recognizable.

At 58 hr. some Rohon-Beard cells within the cord have already formed longitudinal fibres (Text-fig. 3), which constitute a tract extending over the dorsal quadrant of the cord. They are noticeably larger in calibre than the fibres of the longitudinal ventral motor system. As yet, these two tracts are separated by a dorsolateral sector of the cord still uncovered by fibres.

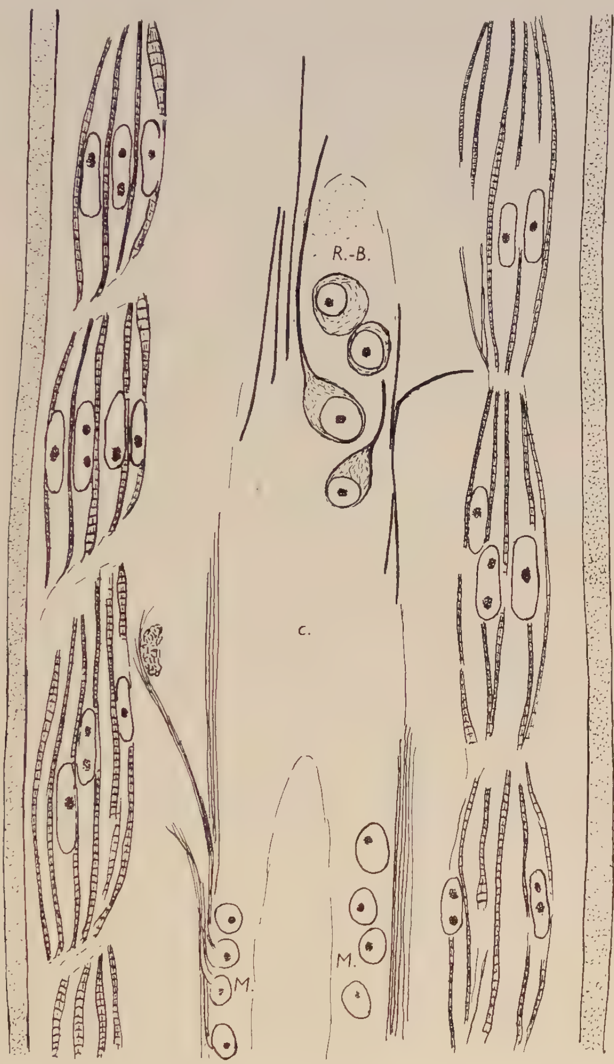
In *Xenopus*, among the logitudinal fibres of this first sensory tract, some run rostrally for short distances, and then turn sharply through a right-angle as they leave the cord (Text-fig. 1 B). These lateral branches then run downwards between cord and myotome, usually near the mid-point of the latter.



Text-fig. 3. Near-horizontal section of a *Xenopus* larva at 58 hr. The cord (*c.*) is cut at an acute angle. A group of Rohon-Beard cells (*R.-B.*) are shown, from some of which longitudinal ascending fibres arise. Dorsal to the cord is an extra-medullary neurone (*E.N.*) whose dendrite runs in an intermyotomic septum. *m.*, melanoblasts.

After 100 hr. of development, further changes in the primary sensory system are evident (Text-fig. 1 C). Dendritic fibres have extended farther, and now plentifully supply both skin and muscle. The lateral branches of those fibres which run in the septa between the myotomes turn forwards to run in intimate contact with the inner surface of the ectoderm. Moreover, at mid-myotome level, lateral branches from

the longitudinal tract now run over the top of the myotomes and then downwards between skin and muscle. Other branches run vertically towards the dorsal fin. Within the cord, cells which send processes directly outwards normally have the axis vertical with the nucleus at the lower pole of the cell (Pl. 1, fig. 3). Only



Text-fig. 4. Near-horizontal section of a *Xenopus* larva at 81 hr. The cord (*c.*) is cut at an acute angle, and the section extends through its upper two-thirds. A group of Rohon-Beard cells (*R.-B.*) is shown with their longitudinal fibres, one of which gives off a lateral branch to an intermyotomic septum. Lower down are seen motor neurones (*M.*), and fibres of the first longitudinal tract, which at this stage are much finer than those of the sensory system.

longitudinal fibres, however, are given off by most Rohon-Beard cells, and in them the axis of the perikaryon may point in any direction. Their fibres may run either rostrally or caudally; sometimes a cell gives off a fibre in each direction. Lateral

branches from caudally directed fibres can be seen ramifying in the septa between myotomes (b_2, Text-fig. 1 C). It is possible that these descriptions may not exhaust all the possibilities of arrangement of cells and fibres at this stage.

The progressive differentiation of the Rohon-Beard neurones involves changes within their perikarya. A Rohon-Beard cell is first recognizable by its shape and size towards the 40th hour of development. The texture of its cytoplasm is then similar to that of other neuroblasts, for all are still heavily loaded with yolk-granules (Pl. 1, fig. 1). The nucleolus is relatively small, and consists largely of Feulgen-positive material.

The Rohon-Beard cells are migrating towards the mid-line at the time when their yolk-granules are being absorbed (Pl. 1, fig. 2). After these cells have reached the median plane, the change in the orientation of the perikaryon is accompanied by a marked increase in total volume. Within the cytoplasm, Nissl substance then becomes apparent. In such amphibian neurones, this material takes the form of scattered granules and rodlets, with a denser layer at the cell surface (Pl. 1, fig. 3). This Nissl material is strongly absorbent at 2537 Å; furthermore, it gives a metachromatic reaction to thionin buffered at pH 3·5 similar to that characteristic of the corresponding material within the Amniote neurone (Windle, Rhines & Rankin, 1943).

Within the nucleus of the mature neurones of *Xenopus*, the nucleolus is very large and spherical and is intensely absorbent at 2537 Å. Its Feulgen-positive material is restricted to a thin and inconspicuous layer at the surface. In this respect, the neurone at this period of development resembles other differentiated cells of *Xenopus* such as myoblasts and melanocytes.

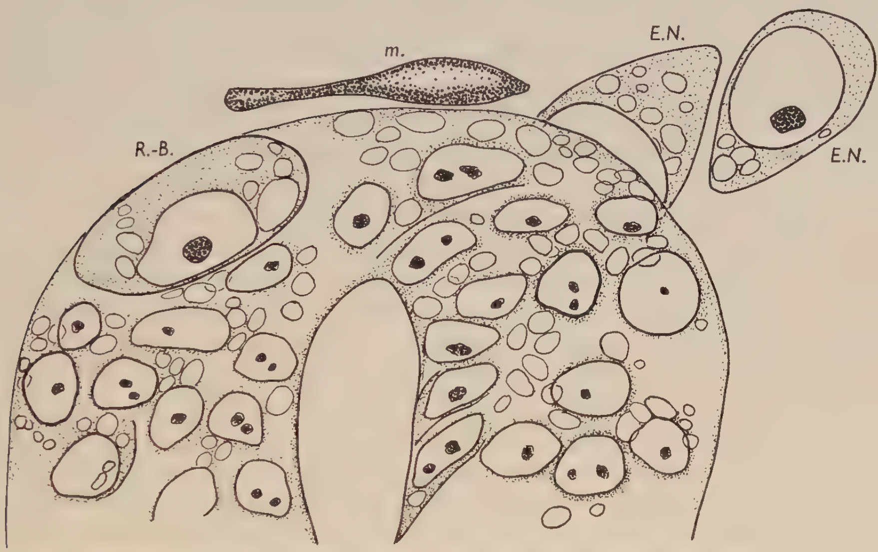
It seems likely that the migration of the Rohon-Beard cells towards the mid-line is merely part of a general movement of tissue within the dorsal part of the cord. While these cells are in a dorsolateral position, the central canal is roofed by single thin elements of the neuro-epithelium (Pl. 1, fig. 1). However, as the Rohon-Beard neurones move to the mid-line, the canal markedly changes in shape, as its upper boundary retreats ventrally. One or two layers of ependymal cells then take up positions between the Rohon-Beard cells and the central canal (Pl. 1, figs. 2–4).

It now remains to compare the Rohon-Beard system of *Xenopus* with that of *Amblystoma*. We see that in both species these cells innervate both skin and muscle, at first by fibres which run in the septa between the myotomes. Again in both, fibres at other levels develop later. The primary sensory systems of these two Amphibia apparently differ in the final pattern of their fibres, for in *Xenopus* there seems to be greater variety in arrangement. In *Amblystoma*, Coghill concluded that all longitudinal fibres running rostrally from the Rohon-Beard cells were axons; he claimed that the longitudinal sensory tract was capable of conduction only in a rostro-caudal direction. In *Xenopus*, however, towards the end of the first week of development it can clearly be seen that lateral processes of Rohon-Beard cells which emerge from the cord may arise from fibres of the longitudinal tract which run in either direction.

(3) *The extra-medullary neurones of* Xenopus

At 58 hr., occasional groups of neuroblasts are to be seen just outside the neural tube, particularly in the cranial part of the cord (Text-fig. 1 B). From some of these, a nerve fibre has already developed which has reached the septum between two adjacent myotomes. Such fibres closely resemble those of the Rohon-Beard cells at this stage (Text-fig. 3). The development of the perikarya, with respect to size and shape of nucleus and cytoplasm, is again similar for neuroblasts in both positions.

Occasionally in a group of these cells, one can be seen half protruding from the neural epithelium (Text-fig. 5) all stages of the apparent migration of neuroblasts from the neural tube may be observed; and it thus seems probable that the extra-



Text-fig. 5. Part of a transverse section through the cord of a *Xenopus* larva at 58 hr., which shows a Rohon-Beard cell (*R.-B.*) within the cord. Two cells of the extra-medullary series (*E.N.*) are seen, one of which is emerging from the cord. Yolk granules within cytoplasm are still present. *m.*, melanoblast.

medullary cells are derived from neuroblasts which differentiate within the cord, and which are at first indistinguishable from Rohon-Beard cells. This view is supported by the fact that while at 43 hr. intra-medullary neuroblasts are clearly recognizable, yet outside the cord the only cells from the neural crest which have then differentiated are the melanoblasts (Text-fig. 2 A). No large cells are then seen which do not contain pigment granules.

By 81 hr., extra-medullary neurones are moving in either of two directions. At anterior levels, some are by now so far lateral that they have approached the dorsal borders of the myotomes (Text-fig. 2 B). At 106 hr., peripheral fibres from these neurones can be traced laterally downwards between skin and muscle, while their central fibres enter the dorsal surface of the cord (Text-fig. 1 C).

The remaining extra-medullary neurones are then migrating towards the mid-line (Text-fig. 2 C). In this plane they take up positions dorsal to the pigmented sheath

of the cord. These cells persist for considerably longer than do their laterally placed equivalents at anterior levels, which are already much rarer by 154 hr. At this stage, medial cells occur sporadically at all levels above the cord. Caudally, they are very large, sometimes with a diameter of a third that of the whole cord. The Rohon-Beard cells at these levels, however, are often not much larger than the surrounding neuroblasts.

The differentiation of the perikarya of the extra-medullary neurones exactly parallels that of the Rohon-Beard cells which has already been described.

THE REPLACEMENT OF THE ROHON-BEARD CELLS BY THE DORSAL ROOT GANGLIA

In *Xenopus* the sensory system of the trunk is provided by the extra-ganglionic neurones for a much greater proportion of the whole period of development than is so in other Anura. This conclusion emerges from a comparison of the time at which the dorsal root ganglia are formed in the several species. From the description of the development of these structures given by Held (1909) in *Rana* it is clear that their anlagen are recognizable before hatching, and that soon afterwards, at the 7–8 mm. stage, nerve fibres are formed by their constituent neuroblasts.

Xenopus at 18° C. hatches at about 72 hr., while with *Rana sylvatica* this period is about twice as long. In *Xenopus*, however, fibres from neuroblasts of the dorsal root ganglia first enter the spinal cord at about 270 hr., at the stage of the 'second-form tadpole' (Weisz, 1945*c*). The Rohon-Beard cells, and the corresponding extra-medullary neurones, thus provide the afferent system of the trunk for about 200 hr. of free-swimming life.

Among the Urodela there seems to be considerable variety in these respects. Thus, while in *Triton* the dorsal root ganglia develop early, at much the same period of development as in *Rana* (Held, 1909), they assume a functional state much more slowly in *Amblystoma* where, as Detwiler (1937) has shown, the spinal ganglia are definite segmental groups of cells by stage 37, but their dorsal roots are not formed until nearly a month later, at stage 45.

In *Xenopus*, the Rohon-Beard cells are not immediately superseded when the neurones of the dorsal root ganglia send fibres into the cord. At 350 hr., the Rohon-Beard cells are still plentiful at trunk levels, though by then only occasional examples are found in the tail. In a 'third-form tadpole' at 760 hr., only three Rohon-Beard cells were found within the whole cord.

During the course of the period of larval life when the dorsal root ganglia are functional, changes within the Rohon-Beard cells are visible. By the 700th hour they sometimes appear shrunken, with an irregular outline to the cell. The Nissl substance in such cells is confined to arcs immediately beneath the cell membrane (Pl. 1, fig. 4). These neurones gradually become similar in size to the neighbouring cells, and often the remaining traces of Nissl substance are the only indication that a cell has once been a Rohon-Beard neurone.

It seems, then, that the Rohon-Beard cells can approximate to the condition of those which surround them. In enumerating Rohon-Beard cells at these stages, it is necessary to fix an arbitrary standard of what is to be counted as such, for their

decrease in size is gradual and progressive. They do not appear to degenerate, and pycnotic nuclei are not seen in the de-differentiation of a Rohon-Beard cell. In late larvae, small dense nuclei are often seen within the cord, but these are found elsewhere than in the mid-dorsal positions, and clearly belong to invading microglial cells. Nieuwkoop & Faber (1956), however, find that during the final stages of reduction of a Rohon-Beard cell (stage 55, ± 32 days) the nucleus becomes pycnotic.

DISCUSSION

In his paper on the later larval stages of *Xenopus*, Weisz (1945*a*) lists no less than twenty features in which the tadpole of this species differs from that of other Anura. Of these features, the most striking is its ciliary feeding mechanism, which has been developed secondarily and independently from the endostylar apparatus of primitive Chordates. With the filter-feeding habit of the *Xenopus* larva are connected other specializations, such as the disproportionately large head, and possibly its neomorphic internal respiratory system.

These features, however, do not exhaust the list of its peculiarities. In the circulatory system, the aortic arches are aberrant; again, the development of the fore-limbs within a closed pouch is not known elsewhere among the Anura.

We must now discuss whether any features in the development of the nervous system which have here been described contribute to this list of the special features of *Xenopus*. It must first be said that any conclusions on such points are highly tentative, for lack of sufficient information on the embryology of other forms. It seems, however, that the pattern of development of the Rohon-Beard cells in *Xenopus* can be paralleled in other Amphibia but, as far as is known, the presence of a system of dorsal extra-medullary sensory neurones has not hitherto been described for any other Tetrapod. Studies on several Amphibia would be needed before this could be stated as a firm conclusion, particularly on the nearest relatives of *Xenopus*, namely the other aglossan genus *Pipa*.

It would seem that the presence of these extra-medullary neurones in *Xenopus* could be regarded as a primitive feature, but this question depends on how far these cells are homologous with the Fritsch cells of fishes. In *Xenopus* their distribution dorsolaterally at cranial levels in the cord strikingly recalls that of the extra-medullary cells in Teleosts such as *Lophius*, but again, a systematic study of the development of these cells in a suitable Teleost is needed. Such a study could be of great value in determining the general homology of these cells in the various orders of fish.

SUMMARY

1. In the larva of *Xenopus laevis*, the primary sensory system of the trunk is made up not only of the Rohon-Beard cells within the cord, but also of extra-medullary neurones, which appear to migrate from the cord at an early stage of neural differentiation.

2. The Rohon-Beard cells of *Xenopus* resemble those of other Amphibia so far as they are known, but extra-medullary neurones do not appear to have been recognized elsewhere among Tetrapods.

3. Descriptions are given of both types of neurone, their migration, the course of their fibres, and of the changes within their perikarya.

4. This primary sensory system is gradually superseded after the dorsal root ganglia have become functional.

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EXPLANATION OF PLATE

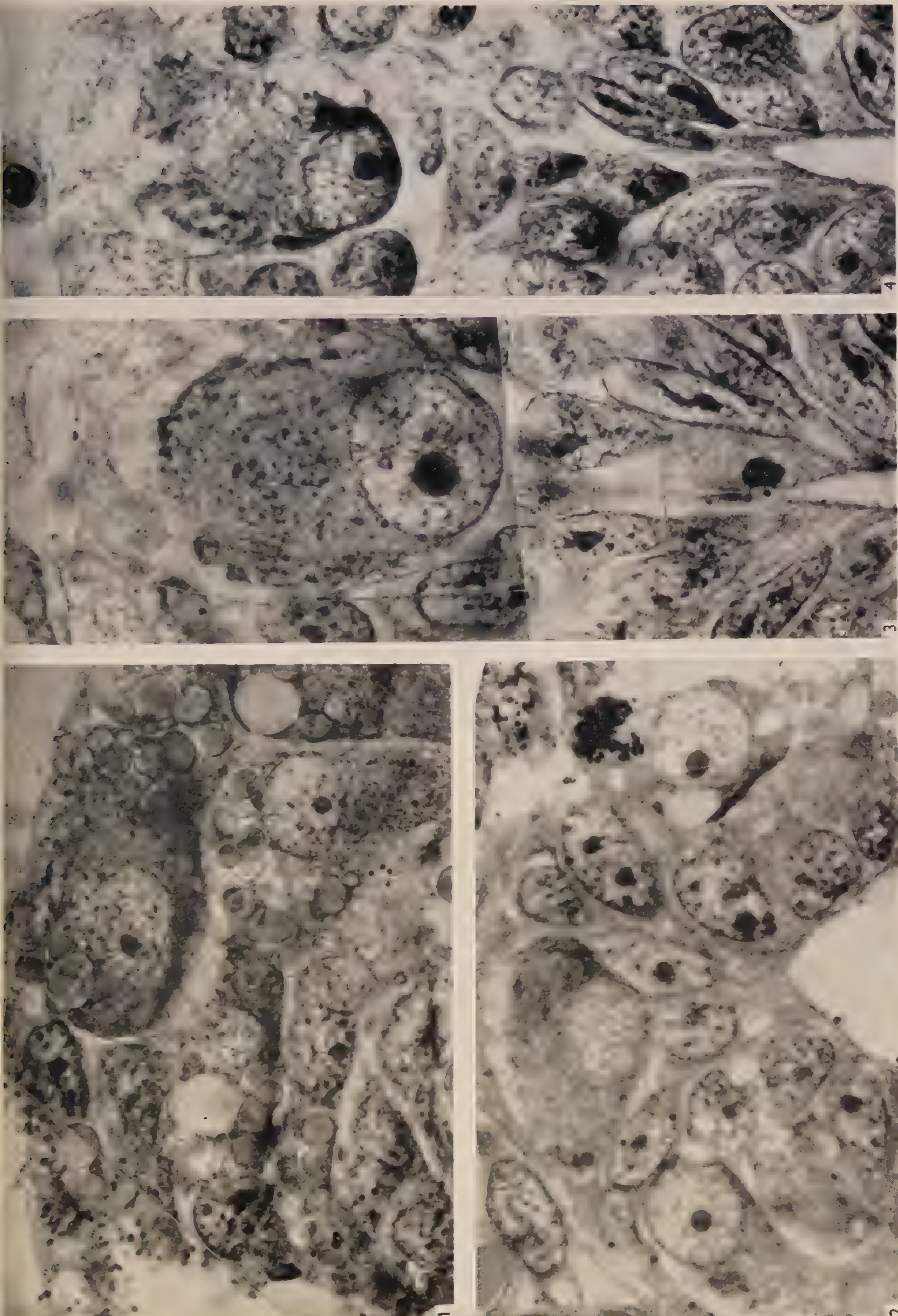
Photomicrographs at 2537 Å of parts of sections through the spinal cord of stages in development of *X. laevis*, to show Rohon-Beard cells. The sections are unstained. All × 1800. (Reduced 10 = 9.)

Fig. 1. 43 hr. (some 15 hr. before hatching). Right-hand upper quadrant of section through spinal cord, with central canal on left; Rohon-Beard cell in dorsolateral position. Yolk spheres are still present in the cells; granules of the original egg-pigment are seen in upper left of figure. The Rohon-Beard cell has a small nucleolus, with as yet no differentiation within the cytoplasm.

Fig. 2. 106 hr., tail region. The lower border of the figure bisects the central canal. The Rohon-Beard cell has moved to a mid-dorsal position. The section misses its nucleolus.

Fig. 3. 252 hr. Mid-dorsal segment of the cord; the lower part of the figure reaches the central canal. A Rohon-Beard cell is mature in size and form, with large, intensely absorbing nucleolus, and Nissl granules within the cytoplasm.

Fig. 4. 298 hr. By this stage the dorsal root ganglia have become functional. Mid-dorsal segment of cord; the lower part of the figure reaches the central canal. Early phase of regression of Rohon-Beard cell, with shrunken cell and nuclear membranes. The Nissl substance is now concentrated near the nucleus.



HUGHES—Development of the primary sensory system in *X. laevis*

(*Facing p.* 338)

EXPERIMENTAL CORNEAL HOMOGRAFTS

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The immune concept which has dominated work on grafting problems in recent years has tended to obscure an aspect of tissue transplantation which is implicit in the results of the extensive experimental work of Loeb (1945) in this field. Loeb emphasized the variation in the behaviour of tissues and organs both as autografts and as homografts. Thus the tissues of a skin autograft survive, but those of a bone autograft are replaced by new bone formed in part from surviving elements of the graft and in part from metaplasia of host tissue. As is well known, the conducting components of a nerve autograft degenerate and only the stromal framework survives, while the functional activity of glandular grafts depends on the physiological conditions of the experiment. There is also variation in the behaviour of different tissue homografts with a wide range of survival period, from a few days to a year or more. Skin homografts and most other tissues are destroyed within 1–2 weeks, but in certain circumstances homogenous bone-marrow cells survive within the host (Ford, Hammerton, Barnes & Loutit, 1956), and the behaviour of homografts of endocrine glands can be influenced by removing the corresponding glands from the host animal (Woodruff & Woodruff, 1950; Deansely, 1954).

There is experimental evidence that spleen homografts (Knake, 1955) and subcutaneous adrenal homografts (D'Arcy, 1952) can live up to 7 months, and grafting of homogenous cartilage and cornea which survive for long periods are established clinical procedures.

We have, however, no knowledge of any record of results of heterotopic transplantation of homografts of whole cornea, and this work describes the fate of corneal homografts implanted subcutaneously and examined at intervals up to 4 months. As clinically a second corneal homograft may be necessary when for technical or other reasons a first attempt has failed, the fate of second-set subcutaneous corneal homografts—in each case from the same donor as the first graft—is considered important and so is included in the experiment.

MATERIAL AND METHODS

The grafts were prepared from the anterior part of the guinea-pig eye, by cutting through the limbus corneae with a sharp razor, taking particular care to avoid any damage to the cornea itself. Thereafter the whole corneae were inserted into the subcutaneous tissues of the lateral body wall of adult guinea-pigs of known mixed stock. There were three experiments:

(1) A corneal homograft was left in the subcutaneous tissue of each of twenty host animals for 21 days. 24 hr. before the grafts were removed the animals were given an injection of 5 mc. of $^{35}SO_4$ at pH 5, and the hosts' corneae and trachea were removed along with the graft to provide controls.

(2) Twenty homografts were implanted subcutaneously, one in each animal, and groups of five were later removed at monthly intervals from 1 to 4 months.

(3) In fifteen animals a corneal homograft was inserted into the subcutaneous tissue of the left lateral body wall, and 4 weeks later a second corneal homograft from the same donor was placed in a corresponding position on the right side and the two grafts left in position for a further 3 weeks. 24 hr. before removal the host animal was given 5 mc. of $^{35}SO_4$. First- and second-set homografts were removed along with portions of the host's cornea and trachea.

The grafts were fixed for 1 hr. in a mixture (3 to 1) of absolute alcohol and acetic acid and subsequently for 24 hr. in formol saline. 10 μ sections of all the homografts were stained either with H. and E. or 0·5 % aqueous solution of toluidine blue. In addition, in Exps. 1 and 3, 5 μ sections were cut of the homografts, the host cornea and the trachea. A number of sections from each block were prepared for autoradiography by the stripping film technique (Pelc, 1947), with an exposure of about 42 days. After development, fixation and drying, the autoradiographs were photographed.

DESCRIPTION

Experiment 1

Seventeen of the grafts were recovered, three having been absorbed. Fourteen of these grafts were in the form of a cyst (Pl. 1, fig. 1). The tendency of subcutaneous corneal grafts, like subcutaneous skin grafts, to form inverted cysts has already been noted (Wyburn & Bacsich, 1952). The cysts varied in size, were transparent or translucent, and usually contained fluid. The grafts were removed without difficulty, and microscopically the only evidence of any host reaction was an occasional mild lymphocytic infiltration. Corneal epithelium formed a lining for the fourteen cystic grafts and showed good histological preservation (Pl. 1, fig. 2). In some grafts the five layers of the epithelium could be recognized, but in most of the cysts the structure of the epithelium varied in different regions. In the large cysts conjunctival epithelium, recognized by the prominently stained goblet cells, completed the lining. In the three non-cystic grafts there was no corneal epithelium. The tissue forming the main mass of the recovered grafts was corneal ground substance showing the characteristic metachromatic staining with Toluidine blue which distinguishes it from any included scleral tissue. Compared with the normal corneal ground substance it was broader and rather more cellular, probably due to a proliferation of graft stromal cells (Pl. 1, fig. 2). No corneal endothelium could be recognized in the graft sections, but Descemet's membrane was present, situated on the outer circumference of the cyst (Pl. 1, fig. 1). There was no evidence in any of the grafts of invasion or destruction of the ground substance by host cells.

Pl. 2, fig. 9, shows autoradiographs of the grafts and the host cornea. It is assumed that the uptake of $^{35}SO_4$ indicates a living tissue actively concerned in the metabolic cycle of the breakdown and new formation of the mucoitin sulphate of the corneal ground substance (this should be compared to the autoradiographs of cartilage homografts (Wyburn & Bacsich, 1955)). There was little difference between the autoradiographs of normal and graft corneae, indicating that the graft had established adequate nutritional relations with the host and was active in the turnover of ground substance.

Experiment 2

At the end of the first month all five grafts were recovered—four had formed cysts and the fifth was a flattened disk. Corneal epithelium lined the four cysts as in Exp. 1, and in all five the corneal ground substance was normal and stained metachromatically with toluidine blue. There was no evidence of host-tissue reaction.

At the end of the second month four of the five grafts were recovered, and the site of the fifth graft was indicated by scar tissue and scattered pigment cells, probably retinal pigment cells incorporated in the graft (Wyburn & Bacsich, 1952). Three of the four grafts were cystic and lined with corneal epithelium, and in all four the ground substance was normal and retained its metachromasia (Pl. 1, figs. 5, 6). At the end of the third month only two of the five grafts were recovered as small cysts enclosed in a connective tissue capsule. The cysts were lined by a single layer of flattened cells and not by stratified epithelium, and the ground substance, although more cellular than normal, was still avascular and stained metachromatically.

No grafts were recovered after 4 months. The site of implantation was indicated by pigment cells, and in two animals small flattened disks were found which in section showed increased metachromasia compared to the surrounding connective tissue.

The significant findings in this experiment are: (1) the preservation of the corneal epithelium lining the graft cyst at the end of three months—considerably longer than the expectation of survival of the epithelium in skin homografts, and (2) the state of the ground substance still unvascularized in its heterotopic position at the end of three months.

Experiment 3

The first set of fifteen homografts were 7 weeks and the second set 3 weeks old. Nine of the first set of grafts were recovered and twelve of the second set, which, according to the result of Exp. 2, is what would be expected in the absence of sensitization. Table 1 summarizes the results of this experiment, and it should be noted that there was no correlation between the survival or absorption of the first and the

Table 1. *The fate of first and second crops of subcutaneous corneal homografts*

	First graft		Second graft	
Animal no.	Corneal epithelium	Corneal Ground substance	Corneal epithelium	Corneal ground substance
228	+	+	+	+
229	+	+	−	+
230	−	+	−	+
231	+	+	−	−
232	+	+	+	+
233	−	−	+	+
234	−	−	+	+
235	+	+	+	+
236	+	+	+	+
237	−	+	+	+
238	+	+	−	−
239	−	−	−	−
240	−	−	+	+
241	−	−	−	+
242	−	−	−	+

corresponding second set homografts. Of the nine first-set homografts recovered, seven were cystic and had a lining of corneal epithelium. In all nine grafts the appearance of the ground substance resembled that of the 2-month-old homografts of Exp. 2. Eight of the recovered second-set homografts had formed cysts of the usual size range, and four were flattened disks. Histological examination showed no unusual host tissue reaction—only a thin connective tissue capsule and a few scattered lymphocytes. All eight cysts had a lining of corneal epithelium varying in thickness in different grafts and in different regions of the same graft (Pl. 1, figs. 3, 4). There was no evidence of degeneration or sloughing of cells, nor was there any significant infiltration of host cells within the cysts. The ground substance of the 12 sec. set homografts was somewhat thicker and more cellular than that of the host cornea, but was otherwise normal and stained metachromatically with Toluidine blue. There were no necrotic areas or regional invasion by host cells.

Autoradiographs of the second set of grafts resembled those of the host cornea and first-set homograft of Exp. 1 (Pl. 2, fig. 10), with no evidence of diminished uptake of $^{35}SO_4$. This is interpreted to imply that the graft cells of the corneal ground substance are functioning normally and utilizing the $^{35}SO_4$ to synthesize the mucoitin sulphate required for the removal of ground substance.

DISCUSSION

These results confirm that cornea, like cartilage, behaves as a homograft quite differently from most other tissues, and can survive well beyond the period of 2–3 weeks within which an immune response normally destroys antigenic tissues. The corneal homografts which formed inverted cysts retained a lining of corneal epithelium, but cystic formation does not by itself confer protection from a host reaction as the behaviour of skin homografts which form cysts when implanted subcutaneously is identical with that of orthotopic controls (Medawar, 1948), and after 3 weeks there is no evidence of lining epithelium (Scothorne, 1957). Our earliest observations are on three-week-old grafts, by which time there is no epithelium in the non-cystic grafts. At the present time we do not know how long this epithelium survives or how it is removed and can only record that in corneal homografts the epithelium does not survive for as long as 3 weeks when it is in direct contact with the host tissues. In these cases, however, there was no evidence of any vascularization or invasion of the corneal ground substance.

When the corneal graft forms a cyst the epithlium is preserved for periods up to 3 months, and in the long-term grafts, as in the short-term non-cystic ones, the loss of corneal epithelium was not associated with the leukocytic, lymphocytic or fibroblastic infiltration normally found during the destruction of unacceptable homografts, for example, skin or nerve.

Billingham & Boswell (1953) transplanted homografts of corneal epithelium, stripped of all but a thin layer of ground substance, to a prepared area on the body surface of rabbits, and recorded the destruction of these corneal epithelium grafts in from 13 to 18 days. This confirms our opinion already expressed elsewhere (1947, 1954), that the ground substance is necessary for the normal nutrition of corneal epithelium.

The absence of the usual host response to foreign tissue could mean that there has been no antibody formation, possibly because the ground substance forms a physical barrier to the passage of antigens into the host tissue. Following skin homografts there is an accumulation of characteristic large lymphoid cells in the regional lymph nodes of the host (Scothorne & McGregor, 1955), which it is considered are concerned with the formation of antibodies. Craigmyle (1955, 1956) reports that after cartilage and corneal homografts there is little evidence of a response in the host's regional lymph nodes. On the other hand, the ground substance could prevent the penetration of antibody molecules into the graft tissues. In this connexion some recent work is of interest. Prehn, Algire & Weaver (1955) have demonstrated experimentally that transplanted homogenous cells remain viable if they are protected by a diffusible membrane impermeable to host cells.

Where a corneal homograft becomes vascularized, which is not necessarily the result of an immune reaction as autografts in heterotopic situations may also become vascularized, the nature of the ground substance is changed, probably by a depolymerization of the high-grade polysaccharide molecule and the physical barrier is broken down.

Billingham & Boswell (1953) found there was a more rapid destruction of their second-set corneal epithelium homografts from the same donor as the first graft. In the present experiment there is no evidence that the first corneal homografts in any way affected the fate of the second set of homografts. Absorption, survival as a cyst with intact epithelium or in non-cystic form with an actively functioning ground substance was uninfluenced by, and quite independent of, the behaviour of first-set homografts (Table 1).

It is now generally assumed that in the clinical corneal homografts, as in our non-cystic subcutaneous grafts, the epithelium disappears but is replaced by a spread of host corneal epithelium. This, however, is quite different from the course of events in the skin homograft, which sloughs off leaving a raw area. The corneal homograft is clinically a success, the skin homograft is not, and the difference, we believe, is a function of the physical and chemical properties of the corneal ground substance.

SUMMARY

1. Subcutaneous corneal homografts show no evidence of destruction by a host reaction after 21 days. Where the grafts form cysts the corneal epithelium is preserved, and in all recovered grafts the corneal ground substance is histologically normal and functionally active as tested by the uptake of labelled $^{35}SO_4$.

2. Subcutaneous corneal homografts can be recovered up to 3 months after implantation. Where they are cystic the corneal epithelium is present in 3-month-old grafts, and the ground substance can be recognized histologically up to 3 months.

3. There is no evidence of donor-recipient reaction affecting a second-set corneal homograft from the same donor as the first set of grafts.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. A 21-day-old cystic corneal homograft. ×18.

Fig. 2. A 21-day-old cystic corneal homograft showing epithelium and ground substance. ×135.

Fig. 3. A 21-day-old second-set cystic corneal homograft. ×18.

Fig. 4. A 21-day-old cystic second-set cystic corneal homograft showing epithelium and ground substance. ×135.

Fig. 5. A 2-month-old cystic corneal homograft. ×18.

Fig. 6. A 2-month-old cystic corneal homograft showing epithelium and cellular ground substance. ×135.

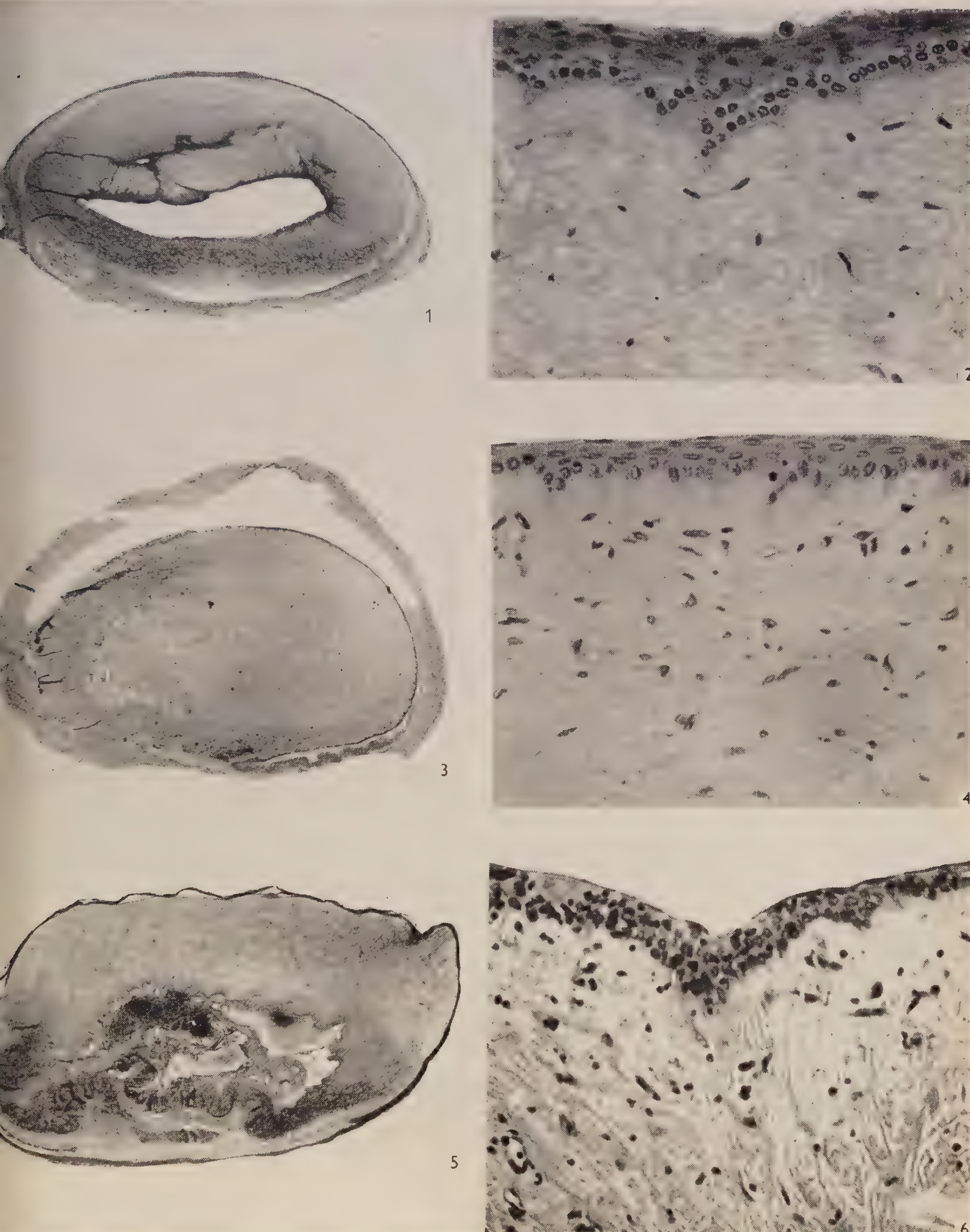
Plate 2

Fig. 7. A 3-month-old cystic corneal homograft. ×18.

Fig. 8. A 3-month-old cystic corneal homograft showing cellular ground substance. ×135.

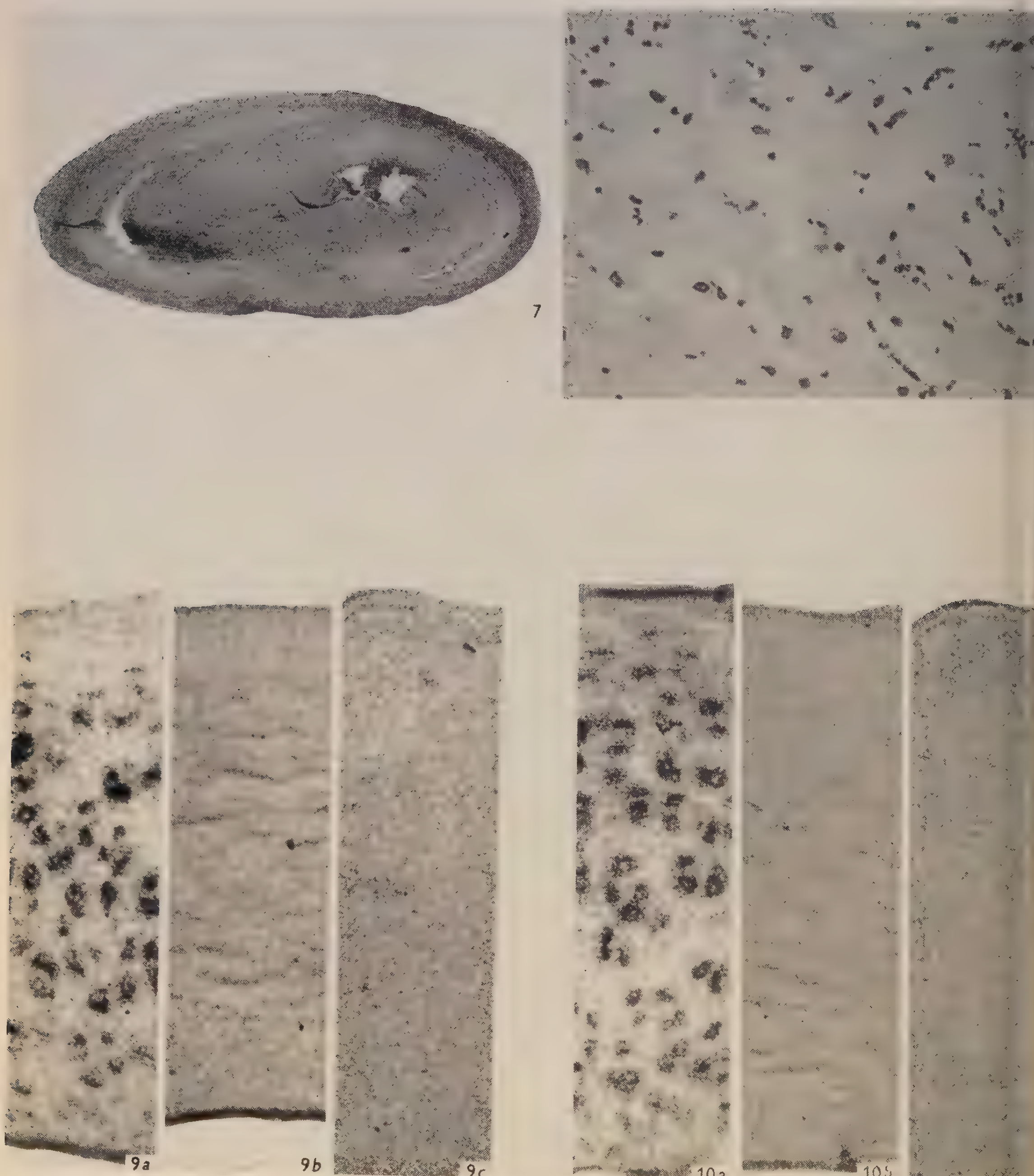
Fig. 9. Autoradiographs. ×180. (*a*) Cartilage from the host trachea. (*b*) Host cornea. (*c*) A 21-day-old corneal homograft.

Fig. 10. Autoradiographs. ×180. (*a*) Cartilage of host trachea. (*b*) Host cornea. (*c*) Second-set corneal homograft.



BACSICH AND WYBURN—EXPERIMENTAL CORNEAL HOMOGRAFTS

(*Facing p.* 344)



BACSICH AND WYBURN—EXPERIMENTAL CORNEAL HOMOGRAFTS

THE MUSCULATURE OF THE HUMAN PROSTATIC URETHRA

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INTRODUCTION

Henle (1866) described two distinct sphincters in the wall of the prostatic urethra. The proximal, composed entirely of smooth muscle, was continuous above with the circular muscle layer of the bladder and formed a complete ring around the internal urinary meatus. He named it the 'sphincter vesicae internus'. Distally, and overlapping the lower fibres of the internal sphincter on their outer surface, he described a 'sphincter vesicae externus', which lay mainly anterior to the urethra, and which near the apex of the gland blended with the deep transverse muscle of the perineum, completely surrounding the urethra. The fibres of the external sphincter were mainly striped, and originated in the stroma of the anterior parts of the prostate gland.

Subsequent workers have generally agreed with the above findings, although there is disagreement on the more detailed anatomy of the muscles (Walker, 1906; Young & Wesson, 1921; Gräning, 1936; Macleod, 1942; and others).

During a previous study of the blood vessels of the prostate gland (Clegg, 1956) it was noted incidentally that, contrary to Henle's view, comparatively few transverse fibres, either of muscle or connective tissue, were seen anterior to the urethra, although posteriorly a thick band of transversely running fibres was observed. In addition, the number of longitudinally directed fibres, especially in the anterior and lateral urethral walls, was much greater than Henle's description warranted. It seemed desirable therefore to re-investigate this problem.

MATERIALS AND METHODS

Prostate glands were removed from fresh post-mortem material. The ages of the subjects, seven in number, ranged from 53 to 82 years. None of the glands showed significant pathological changes, other than small adenomata; these did not affect the findings.

The glands, together with adjoining portions of the bladder, were fixed for periods from 2 to 6 months in 10 % formol saline. In six, transverse sections 50 μ thick were cut on the freezing microtome, and one section in ten was stained by Masson's trichrome method. The remaining gland was sectioned longitudinally and treated in the same way. In a few instances, sections were stained by Mallory's method, but this proved unsatisfactory.

RESULTS

Striped muscle

In no case is striped muscle seen to enter into close relationship with the urethra except near the apex of the gland, where bands of fibres infiltrate the smooth muscle anterior, lateral and sometimes posterior to the urethra. Striped muscle fibres make

their appearance in the anterior region of the gland at the level of the termination of the ejaculatory ducts and progressively increase in number towards the apex.

Smooth muscle

With regard to the arrangement of its smooth muscle, it is convenient to consider the prostatic urethra in three parts, *a proximal* immediately below the internal urinary meatus, *an intermediate*, comprising that part above the opening of the ejaculatory ducts, and *a distal* part extending to the apex of the gland.

Proximal part

In this section of the urethra the main feature is the thick band of muscle running obliquely downwards and medially behind the lumen (Pl. 1, fig. 1). The considerable amount of connective tissue which separates its individual fibres gives it an 'onion-skin' appearance, and it will subsequently be referred to as the 'concentric muscle'.

Its fibres are derived from two sources. Peripherally they have their origin in the muscular stroma of the antero-lateral part of the prostate gland and its capsule. Centrally they are continuous with the transverse fibres of the trigone and with the anterior longitudinal muscular layer of the bladder. Some of these latter fibres pass directly backwards lateral to the internal urinary meatus. Others pass downwards in the anterior urethral wall (Pl. 1, figs. 2, 3) for varying distances before passing obliquely downwards and backwards to the posterior aspect of the urethra.

From the trigone a number of longitudinally directed fibres pass downwards immediately deep to the mucous membrane of the posterior urethral wall (Pl. 1, fig. 3). They are of smaller diameter than the fibres of the concentric muscle.

In the lower part of this segment of the urethra a few transverse fibres, again of small diameter, may be seen in the submucosa of the anterior urethral wall. Some of these are continuous with the innermost concentric fibres, and thus a complete although very thin ring of smooth muscle passes around the urethra a short distance above the crest. There is no anatomical sphincter immediately below the bladder neck, except in the sense that the peripheral part of the concentric muscle is in continuity with the capsular fibres of the prostate, some of which cross the midline anterior to the urethra. These anterior transverse fibres, however, are not in such intimate relationship with the urethra as are the concentric fibres, as they are separated from it by the fibres derived from the anterior longitudinal layer of the bladder.

Intermediate part

The urethral crest is a fibromuscular structure containing the ducts of prostatic glands which open on its sides. The musculature consists of bundles of fibres separated by connective tissue, which run downwards in the line of the urethra. They are in the main continuous with the posterior longitudinal fibres of the proximal part of the urethra, but in addition longitudinal fibres anterior to the urethra, which run obliquely round to its posterior aspect, and a few fibres from the concentric muscle contribute to the muscle of the crest.

As the height of the crest increases, the ejaculatory ducts and prostatic utricle pass forwards and downwards through the substance of the prostate towards the base of

the crest. The ducts and utricle are contained in a sheath, the composition of which changes proximo-distally. Above, it consists mainly of connective tissue with a small number of smooth muscle fibres (Pl. 1, fig. 4). The walls of the ejaculatory ducts contain smooth muscle, but the utricle in five of the six glands sectioned transversely contains no muscle. In the remaining gland, the utricle wall contains well-defined bands of circular muscle (Pl. 2, fig. 5). Below the point where the ducts and utricle turn almost directly forwards the sheath contains considerable amounts of muscle, in which longitudinal, circular, and oblique fibres may be seen, although separate layers cannot be distinguished (Pl. 2, figs. 6–8). This muscle is quite distinct from that of the walls of the ejaculatory ducts.

As the ducts pass forwards into the crest, there lies behind them in the median plane a thick bar of muscular tissue which blends anteriorly with the posterior part of the sheath and which posteriorly appears to be continuous with the concentric muscle. In the crest, this bar, together with the posterior half of the sheath, assumes a 'Y' shape (Pl. 3, fig. 9). The limbs of the 'Y' extend to the urethral submucosa, and the crest is thus divided into an anterior part, containing the terminations of the ejaculatory ducts and utricle, and a posterior, divided by the stem of the 'Y' into two halves, each containing the ducts of the prostatic glands.

At this level the amount of smooth muscle in the submucosa of the anterior urethral wall increases. Both longitudinal and transverse fibres are present, the latter being especially prominent opposite the openings of the ejaculatory ducts and prostatic utricle (Pl. 3, fig. 9). They are continuous with the transverse fibres noted in the proximal segment.

The concentric fibres in the upper part of this segment form a thick mass behind the urethra. As the ejaculatory ducts and prostatic utricle pass forward to their terminations they traverse this muscle. That part anterior to the ducts terminates at or above the level of their entrance into the urethra (Pl. 3, fig. 10). The much thinner posterior part generally persists, although in an attenuated form, into the next segment of the urethra.

Distal part

Immediately below the level of entrance of the ejaculatory ducts and prostatic utricle into the urethra the muscle of the crest still retains its 'Y' shape. With the gradual disappearance of the utricle and ducts, the limbs of the 'Y' approach one another and fuse in the midline, the anterior end of the bar so formed being immediately deep to the urethral submucosa (Pl. 3, fig. 11). This bar persists, although of diminishing height, to the lowest part of the prostatic urethra (Pl. 3, fig. 12).

In this part, the amount of circular muscle surrounding the urethra increases considerably (Pl. 3, figs. 11, 12). In five cases out of seven it is directly continuous with the concentric muscle referred to above, although the connexion immediately below the openings of the ejaculatory ducts is but a tenuous one. The fibres of the muscle arise entirely in the stroma of the prostate in the antero-lateral region, generally a short distance from the mid-line. As noted previously, striped fibres are present in this segment of the urethra. They are most numerous anteriorly, but a few pass backwards to the lateral and occasionally the posterior aspects of the urethra.

DISCUSSION

The smooth muscle of the prostatic urethra

Apart from Griffiths (1891) and Scher (1950) who considered that there was no sphincter at the bladder neck (although the latter author considered that the fibro-muscular tissue of the prostate as a whole could act as one), all previous authors are agreed that some type of sphincter muscle exists in this position.

Most workers agree with Henle (1866) that the bladder neck sphincter is continuous above with the circular muscle of the bladder and that its fibres pass obliquely downwards and forwards on either side of the internal urinary meatus to become continuous with one another anterior to the urethra. Pettigrew (1867), von Ebner (1902), Eberth (1904) and Young & Wesson (1921) were of this opinion, and Griffiths (1889) stated that circular muscle fibres from the bladder could be traced into the urethra, but Walker (1899) and McCrea (1926) considered that these fibres ended at the internal urinary meatus. Testut & Latarjet (1949) regarded the 'smooth sphincter' at the bladder neck as resembling an inverted cone, continuous with the bladder musculature, and traversed from base to apex by the urethra. They considered it to be histologically distinct from the bladder musculature.

Lowsley (1930) and Gräning (1936) found that fibres from the anterior longitudinal muscle layer of the bladder pass round the urethra in a postero-inferior direction; these fibres were continuous with those of the opposite side behind the urethra.

The present findings agree with the last two authors in that the main musculature of the upper part of the prostatic urethra lies behind the urethra, and is partly derived from the anterior longitudinal muscle of the bladder. Its continuity with the transverse muscle of the trigone is also in agreement with Gräning (1936). Thus there appears to be no anatomical sphincter at the bladder neck. From the functional point of view, however, such a muscle as the one described would have the effect of approximating the posterior to the anterior urethral wall, not only at the bladder neck but throughout the extent of the prostatic urethra, and it could therefore close the latter.

The description of the structure of the urethral crest differs from that of Henle (1866) and Testut & Latarjet (1949). These authors described a mass of cavernous tissue lying immediately lateral to the fibromuscular 'skeleton' of the crest. The present findings do not substantiate this.

The striped muscle of the prostatic urethra

The extent to which voluntary fibres are concerned in the formation of the urethral musculature is in some dispute. It is agreed that the amount of striped muscle increases towards the apex of the prostate, but some authors consider that it is present in the upper part of the prostatic urethra. McCrea (1926) maintained that striped fibres were present in the trigone, Henle (1866), Walker (1906) and Young & Wesson (1921) found that the sphincter muscles at the bladder neck had a striped component, while Testut & Latarjet (1949) found fibres of their 'striped sphincter' as high as the neck of the bladder.

In the present investigation no striped muscle fibres were present in the urethra

above the level of entrance of the ejaculatory ducts and prostatic utricle. The whole extent of the trigone of the bladder was never examined, but in no case were striped fibres seen in this situation. However, below the level of the entrance into the urethra of the ejaculatory ducts and prostatic utricle the number of striped muscle fibres progressively increases.

Henle and most of the subsequent writers on the subject consider that a distinction can be made between the internal and external vesical sphincters both in their constitution and in their anatomical identity. The present findings do not completely substantiate this view. Although the muscle near the apex of the gland which corresponds to Henle's 'sphincter vesicae externus' contains striped fibres, its smooth component is similar in origin and distribution to the smooth muscle fibres in the upper part of the urethra (the fibres corresponding to Henle's 'internal sphincter') and the two groups of fibres are usually contiguous, although the muscle is always thinner below the openings of the ejaculatory ducts, and in a minority of cases an actual gap is present. In addition, the striped fibres are comparatively small in number and probably have little sphincteric action. It would seem more accurate, therefore, to regard the urethral sphincter as a single muscle which has a striped component below the openings of the ejaculatory ducts and prostatic utricle.

The muscle in relation to the terminations of the ejaculatory ducts and prostatic utricle

Little attention has been paid to the muscular arrangements in this situation. The presence or absence of muscle in the wall of the utricle has caused controversy; thus Walker (1899), von Ebner (1902), Eberth (1904) and Testut & Latarjet (1949) consider that it is present, while Stieve (1930) denies its existence. The present findings indicate that the muscle is more often absent; it may be that previous workers confused muscle in the common sheath of the duct and utricle with muscle in the wall of the utricle.

With regard to the ejaculatory ducts, all authors except Eberth agree that muscle is present in the duct walls, but the presence of extra-mural muscle has received little attention. The suggestion that a sphincter may exist at the terminations of the ducts has been made by Finger (quoted by Walker, 1899); Eberth also noted the existence of circular and oblique fibres around the ducts. The present findings indicate that in the lower part of the sheath of the ducts there are both longitudinal and transverse muscle fibres, although proximally the sheath is composed of connective tissue with few, if any, muscle fibres.

It could be contended that these fibres are concerned with the expulsion of the ejaculate, but their localization around the ends of the ducts and their extra-mural situation lead one to the hypothesis that they exert a sphincteric action on the ducts and utricle. Obviously some means of preventing the passage of urine into the ejaculatory ducts is necessary; Lowsley (1912) believed that a valve was present at their mouths. Indirect evidence for the presence of a closure mechanism has been provided by Macmillan (1952), who showed that in the rat ligation of the vas deferens did not affect the rate of disintegration of spermatozoa in the tail of the epididymis.

SUMMARY

1. The results of the examination of sections from seven prostate glands, stained by Màsson's technique, are described.

2. Striped muscle fibres are found in that part of the prostatic urethra below the level of opening of the ejaculatory ducts and prostatic utricle. They increase in number towards the apex of the prostate, but never form a true sphincter within the gland itself.

3. Behind the urethra is a layer of transversely arranged smooth muscle which is thickest above and in front of the ejaculatory ducts. This muscle is continuous with the anterior longitudinal layer of the bladder, with the transverse muscle of the trigone and with the stroma of the prostate. Although not a true sphincter, it is considered that it may act as one.

4. The ejaculatory ducts and prostatic utricle are enclosed in a sheath which near their terminations contains much smooth muscle. It is considered that this muscle may exert a sphincteric effect on the ejaculatory ducts.

I am indebted to Prof. R. G. Harrison for his interest and advice. The sections were prepared by Miss B. Birkett, and the photomicrographs by Messrs L. G. Cooper and A. F. Taunton.

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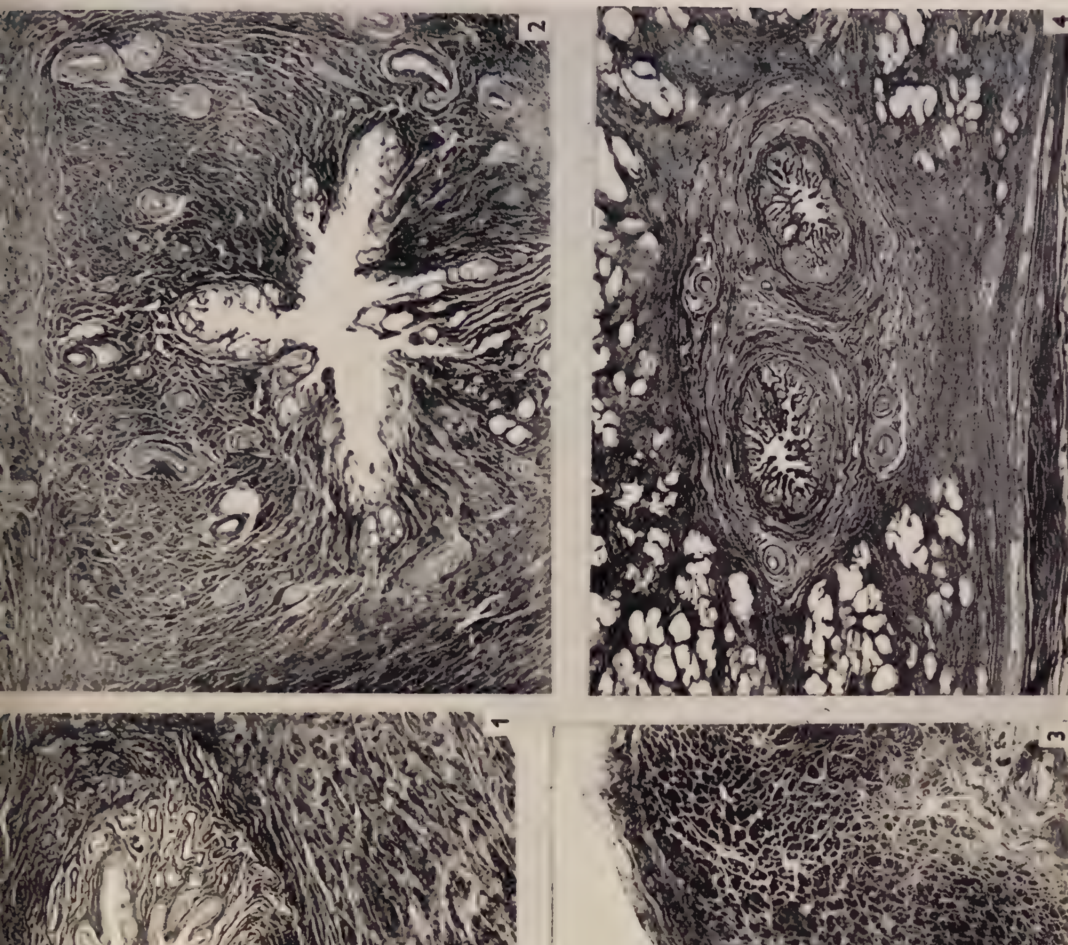
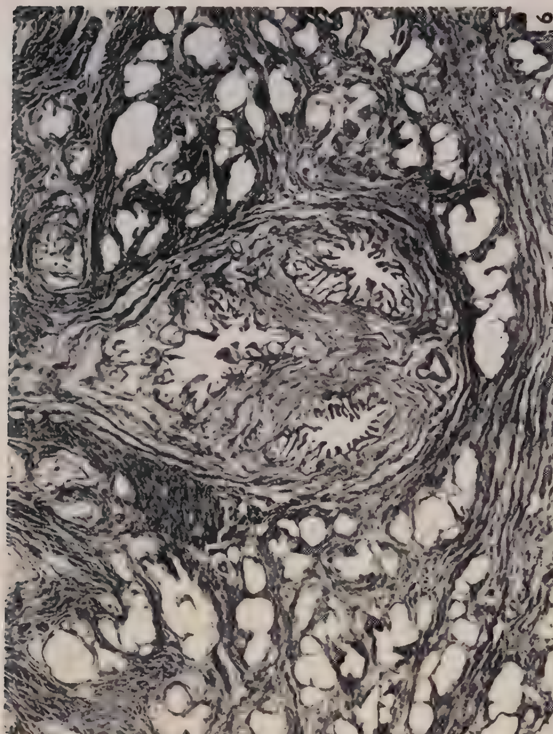
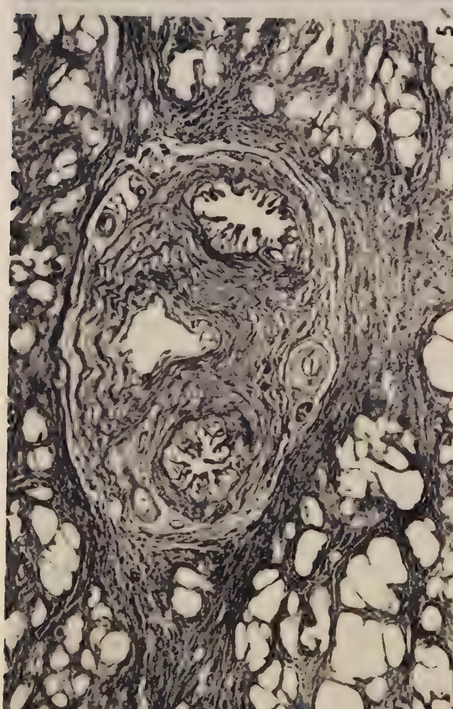
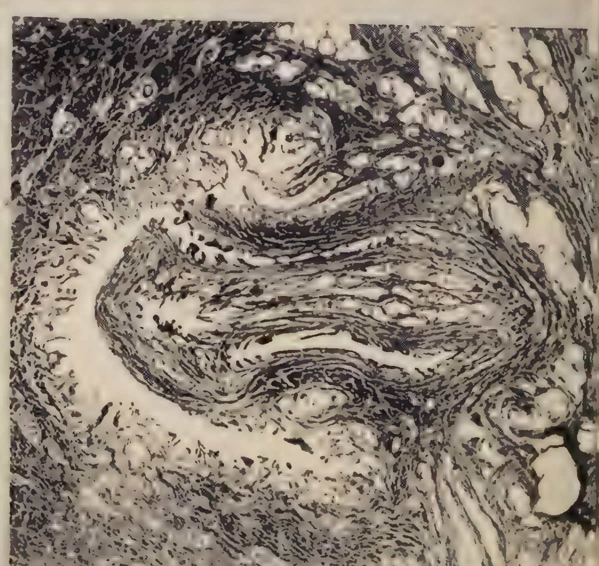


CLEGG—The musculature of the human prostatic urethra

(*Facing p.* 350)







CLEGG—THE MUSCULATURE OF THE HUMAN PROSTATIC URETHRA

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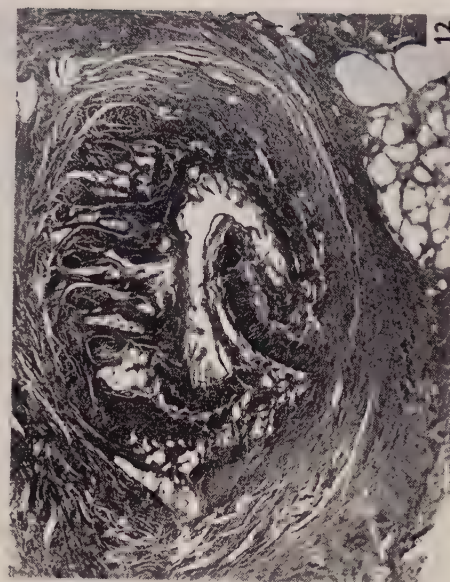

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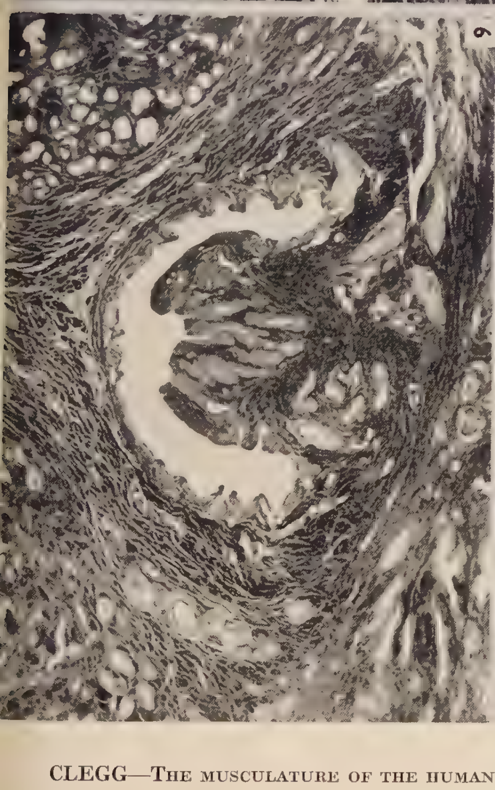

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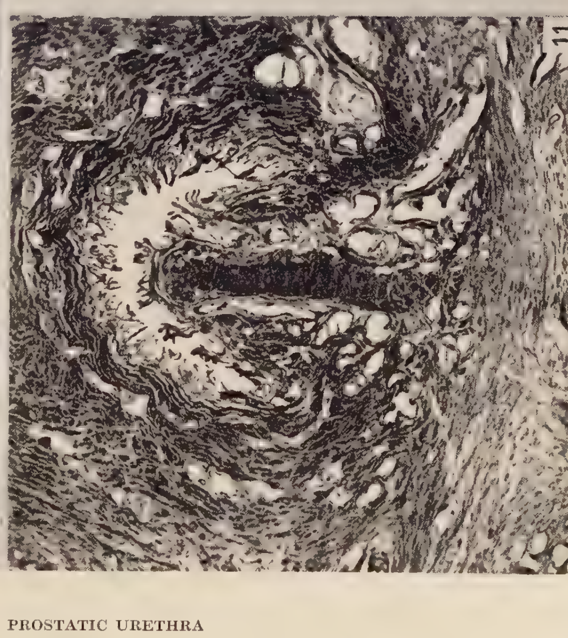

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EXPLANATION OF PLATES

All histological sections shown in the plates were stained with Masson's trichrome stain.

Plate 1

Fig. 1. Transverse section through a prostate just below the internal urinary meatus. The concentric fibres passing behind the urethral lumen can be seen. ×12.

Fig. 2. Transverse section through a prostate a short distance above the urethral crest. The thick bundle of vertically directed fibres anterior to the urethra can be seen. ×10.

Fig. 3 Longitudinal section through the internal urinary meatus. Both anteriorly and posteriorly bundles of longitudinal fibres descend from the bladder into the urethra. They are very thick in front; behind they are much less well developed. The concentric muscle is seen posteriorly. The transversely directed fibres seen anteriorly are part of the prostatic capsule. ×12.

Fig. 4. Transverse section through the ejaculatory ducts in the upper part of the prostate. Although their walls contain muscle, the sheath in which they lie is composed almost entirely of connective tissue. ×17.

Plate 2

Fig. 5. At a lower level in the same gland the lumen of the prostatic utricle can be seen to be suronded by darkly staining muscle fibres. The sheath now contains numbers of muscle fibres, which are distinct from the muscle in the duct walls. ×11.

Fig. 6. In this section the ducts and utricle lie behind the urethral crest. Well-defined muscle fibres, in this case mainly circular, may be seen in the sheath. ×12.

Fig. 7. The ducts and utricle have entered the urethral crest, and one of the ducts is opening into the urethra. On either side of the ducts numbers of longitudinally directed fibres may be seen. ×9.

Fig. 8. Longitudinal section through a prostate near the midline. The ejaculatory duct (*ED.*) can be seen passing towards the urethra (*U.*). Near its termination it is surrounded by a mass of muscle fibres (*M.*), the direction of which is oblique. ×12.

Plate 3

Fig. 9. Transverse section through a prostate at the point of opening of the prostatic utricle into the urethra. The 'Y'-shaped bar of muscle can be seen. A well-defined layer of circular muscle fibres lies immediately deep to the mucous membrane of the anterior urethral wall. ×10.

Fig. 10. Longitudinal section through a prostate above the level of entrance of the ejaculatory ducts into the urethra. The concentric muscle can be seen to thin out inferiorly while the posterior longitudinal muscle continues into the urethral crest. ×14.

Fig. 11. Transverse section through a prostate a short distance below the point of entry of the ejaculatory ducts into the urethra. The median bar of muscle is seen. The circular muscle deep to the mucous membrane of the anterior urethral wall is well developed. ×10.

Fig. 12. Transverse section through the apex of the prostate. The median bar is still present, and the concentric muscle forms an almost complete ring around the urethra. ×7.

THE VENOUS DRAINAGE OF THE RAT SPLEEN

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The majority of the work on splenic vascularization has been concerned with its intrinsic or 'separatory' circulation in various experimental animals, and the contributions of Knisely (1934, 1936*a*, *b*), MacKenzie, Whipple & Wintersteiner (1941), Björkman (1947) and Whipple, Parpart & Chang (1954) are the most important in this field. Their conclusions differ, however, as to whether the circulation is 'open', 'closed' or a combination of both.

This paper deals with another aspect of the splenic circulation, namely, the venous drainage of the splenic compartments or segments under normal conditions and also following certain operative procedures and is an extension and elaboration of a note published previously (Braithwaite & Adams, 1956).

Dreyer & Budtz-Olsen (1952) are commonly regarded as being the first workers to describe the spleen as consisting of a series of compartments each with its own vein (whilst carrying out diagnostic splenic venography). Perusal of the literature shows that Kyber (1870) described that the spleen in man, cat, dog, horse and rabbit was divided into several compartments by 'fibrous septa', and he stated that each segment was supplied by its parent artery. This work was later extended by Henschen & Reissinger (1928). Tait & Cashin (1925) confirmed the presence of compartments in the spleen of the dog and cat and showed that stimulation of individual neuro-vascular bundles of separate zones of the dog spleen produced localized contraction of the segment. Additional support for the presence of splenic segmentation is afforded by pathological evidence.

It is the purpose of this paper to describe and illustrate the segments in the rat spleen and to discuss their venous drainage under normal and abnormal conditions in the *living* animal.

MATERIAL AND METHODS

In this series we have studied the spleens of 107 living rats of both sexes whose weights ranged between 200 and 530 g. Using ether anaesthesia the stomach and spleen were delivered through an incision in the anterior abdominal wall and the mesentery between them carefully dissected in order to free the spleen. A small Kodaline film was then placed in position under the spleen.

In the earlier experiments we carried out *retrograde injections* into one of the tributaries of the portal vein. The superior mesenteric vein was the most accessible and hence most frequently used. The injection masses used were micropaque, thorotrast and in a minority of instances diaginol, and the amount introduced varied between 1 and 3 ml. The mass was introduced slowly at minimal pressures. Radiographs of the splenic vessels were taken at various times during injection and also at intervals after its completion. In some instances the veins draining the spleen

were left intact; in others we ligated alternate veins to obtain a more comprehensive picture of their territories of drainage.

In later experiments we carried out direct injection of thorotrast into the splenic parenchyma in amounts varying between 0·06 and 0·36 ml. by a method similar to that first described by Abeatici & Campi (1951) in the dog. We studied the passage of the medium and the time for its disappearance in radiographs taken during and after injection. In some instances all the vessels leaving the spleen were left intact. In others we carried out ligation of one or more of the tributaries of the splenic vein prior to injection to study the collateral circulation in the various zones of the spleen.

In order to eliminate artifacts that may have been caused by our technique, such as handling of the spleen and injection pressure used, we carried out a small series of experiments under similar conditions in the spleens of dead animals.

RESULTS

After describing the normal venous arrangements of the rat spleen, and our confirmatory evidence for the presence of compartments, the normal drainage of the compartments and the effects of its interruption will be described.

(1) *Normal arrangements*

The venous sinusoids of the rat spleen terminate in collecting veins of the pulp which themselves end in the larger trabecular veins (Snook, 1950). These converge to form the main hilar veins of which there are five to seven corresponding to the terminal hilar branches of the splenic artery (Pl. 1, fig. 1); of these the vein draining the middle segment is usually the largest. The hilar veins terminate in the main splenic trunk which by its union with the superior mesenteric vein forms the portal vein.

(2) *Evidence for the presence of compartments*

(*a*) *Macroscopic separation of a splenic compartment.*

Occasionally one of the splenic segments fails to unite completely with the main mass. Pl. 1, fig. 2, illustrates this and also shows the presence of accessory spleniculi along the course of the splenic vein near the lower pole of the spleen. In the recent state the upper segment which is richly vascularized was separated from the remainder of the organ by a well-defined fibrous partition.

(*b*) *Radiological*

Retrograde injection experiments. Pl. 1, fig. 1, shows the results of retrograde injection into the intact spleen.

After the interruption of alternate vascular pedicles, a radiograph taken at an early stage of the injection shows that only the larger venous radicles have been filled in the middle and lower zones, although the mass has entered finer channels in the upper segment (Pl. 1, fig. 3). The avascular zones alternating with the vascularized areas are clearly defined.

If the interval between injection and radiography is longer a more complete filling of the segments occurs and the mass passes through the finest venous radicles into the pulp spaces (Pl. 1, fig. 4); it is clear that this affords more convincing evidence of splenic compartments than the previous specimen, because more time has elapsed

between injection and radiography. It will be noticed that the lower and middle zones are full to capacity and the overflow has tracked along the venous channel linking the two compartments.

The sizes of the individual segments vary, but the middle is usually the largest and Pl. 1, fig. 5, indicates its extent. This was demonstrated by a direct injection into the middle hilar vein.

(*c*) *Direct intrasplenic injections*

The *normal* drainage of the splenic compartments will be described separately.

(1) *Into the upper compartment.* Pl. 2, fig. 1, is a radiograph taken during the injection of 0·18 ml. of thorotrast into the spleen. Some of the mass can be seen leaving the upper hilar vein, and this occurs at the beginning of the injection. A pool of mass is present at the tip of the needle and there has been filling of the larger venous radicles which are localized to the upper segment. The upper compartment is clearly defined and no injection material can be observed in the adjacent one.

The time taken for this amount of injection mass to disappear varied between 10 and 12 min. Pl. 2, figs. 2, 3, are radiographs of the same specimen taken 5 and 10 min. respectively after injection; it will be observed that only a trace of mass remains in Pl. 2, fig. 3.

When a larger amount of material is introduced (0·36 ml.) the hilar vein again drains away some of the medium, but in addition an alternative route is afforded by the intersegmental vein which can be seen issuing from the upper segment at its well-defined lower border (Pl. 2, fig. 4). The latter vessel is a collateral channel responsible for draining an excess of the mass introduced. It will also be noted that in this figure no communication apart from this vein can be seen connecting the adjacent compartments.

(2) *Into the middle compartment.* Pl. 2, figs. 5, 6, are radiographs taken after the injection of small (0·12 ml.) and large (0·36 ml.) amounts of media into the middle segment of the spleen. Pl. 2, fig. 5, shows a localized collection of injection mass, part of which is being drained by the parent segmental vein. Pl. 2, fig. 6, demonstrates that there has been some backflow along the vein immediately proximal to the middle segmental vein which in turn has caused filling of its larger tributaries. It will be noticed that the two segments shown in the figure are quite different in appearance, for in the middle zone indicated by the needle the mass had penetrated through to the pulp spaces, whereas in the adjacent zone the intersegmental vein and its larger tributaries are the only vessels filled. The time taken for the passage of medium from the injection site along its own vein to the vessel immediately proximal accounts for the different appearances in the two zones.

(3) *Into the zone proximal to the lowest compartment* (Pl. 2, fig. 7). An excess of medium (0·36 ml.) has been injected into this area, and the main route of drainage is indicated by the filling of the parent hilar vein. As in the previous specimen there has been some backflow into the two veins and their larger tributaries which lie immediately adjacent to the injected zone. Another feature of the radiograph is the opening up of the intersegmental vein which emerges from the lower part of the injected compartment.

(4) *Into the lowest compartment* (Pl. 2, fig. 8). This radiograph shows similar

features to those previously described, namely, drainage by the parent segmental vein and localization of the injection material in the lowest splenic compartment. There is some filling of the larger venous tributaries in this zone.

Drainage of splenic compartments following venous ligations

Pl. 3, fig. 1, shows the effects of interrupting the upper segmental vein, Pl. 3, fig. 2, the middle segmental vein and Pl. 3, fig. 3, was taken after all the hilar veins had been occluded. A feature common to all is the opening up of the intersegmental channel which is well marked in Pl. 3, fig. 1, and can be seen both above and below the injection site in Pl. 3, fig. 3.

It was necessary to exclude such factors as the effects of manipulation of the spleen and the pressure of the injection used, on the results obtained in these experiments by carrying out a similar technique on the spleen of dead animals. The result is shown in Pl. 3, fig. 4, and it will be noticed that the appearance is quite different from the figures illustrated previously. The mass injected lies in a pool without any evidence of venous filling in the area; furthermore, there has been no escape from the segment into its segmental vein.

DISCUSSION

Our investigation has shown the presence of fairly distinct compartments in the rat spleen and the results are similar to those of Kyber (1870), Henschen & Reissinger (1928), Tait & Cashin (1925) and Dreyer & Budtz-Olsen (1952) in other species regarding the fact that each splenic segment has its own vessels.

The segments in the rat vary between five and seven in number, of which the largest is placed most centrally—its segmental vein is consequently of larger calibre than the other veins leaving the spleen. When injecting only a *small amount of medium* into one of the segments, drainage in every instance occurs immediately through the parent segmental vein, and 10–15 min. later no opacity can be seen in the radiograph. It is possible that splenic segments can act under normal circumstances as separate units when the blood flow to them is not excessive, and our preliminary arterial studies also confirm this concept. When the capacity of a segment is overtaxed, as in the introduction of *excess medium* or alternatively if its normal portal of exit is obstructed, an alternative route of drainage is afforded by the intersegmental vein (Pl. 2, figs. 4, 6; Pl. 3, figs, 1, 2, 3). It is possible in the light of this evidence that this intersegmental channel affords an alternative route when one or more compartments become congested or if any obstruction should occur in any of the tributaries of the splenic vein. It is suggested that the opening up of the intersegmental vein affords evidence that the splenic segments can be brought into functional unity with each other. It is through this channel that communication between the segments is first brought about, and not, as many have inferred, through the pulp spaces. The present findings afford an explanation for the comments of Whipple *et al.* (1954) on recent work on splenic venography. They state that an injected mass 'suffuses' rapidly through the splenic pulp; the spread of medium from one compartment to the next may possibly occur slowly through direct communication between the pulp spaces of adjacent segments, though we have not

been able to demonstrate this in our experiments. It can certainly take place rapidly through the intersegmental vein and thence through its tributaries into the venous radicles and the pulp spaces of the adjacent compartments they drain. We suggest that previous interpretations were based mainly on radiographs of spleens in which the 'suffusion' had been completed and hence gave no indication of the main pathway of spread of the injection material. We have had the additional advantage in our studies of being able to carry out several radiographs at various times on the same animal and this has considerably facilitated our interpretations.

SUMMARY AND CONCLUSIONS

1. The venous drainage of the rat spleen has been investigated by radiographic methods in 107 animals with particular reference to individual compartments.

2. The splenic compartments, five to seven in number, have been demonstrated both by retrograde injections and also by direct intrasplenic injections. Each is normally drained by its own segmental vein.

3. When individual compartments become overloaded by introducing an excess of medium or if their normal portal of exit is obstructed, additional drainage is afforded by the collateral intersegmental vein. It is by this channel that the medium first passes from one splenic compartment to another and not by diffusion through the pulp spaces of adjacent compartments.

4. The bearing of the present findings on the normal functioning of the spleen is discussed briefly.

We are very grateful to Prof. R. G. Harrison for the benefit of his advice and help throughout the course of this study. We are also indebted to Messrs L. G. Cooper, A. Taunton and D. J. Kidd for technical assistance.

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BRAITHWAITE AND ADAMS—THE VENOUS DRAINAGE OF THE RAT SPLEEN

(Facing p. 356)

BRAITHWAITE AND ADAMS—THE VENOUS DRAINAGE OF THE RAT SPLEEN

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Radiograph of hilar veins in normal spleen. ×2.

Fig. 2. Radiograph illustrating partial separation of the upper segment of the spleen and the presence of accessory spleniculi near the lower pole. ×2.

Fig. 3. Radiograph taken at an *early* stage of a retrograde injection, alternate vascular pedicles having been previously interrupted. ×2.

Fig. 4. Radiograph taken at a *later* stage than fig. 3, having previously interrupted alternate vascular pedicles. ×2.

Fig. 5. Radiograph showing the middle segment of the spleen, after a retrograde injection through the middle hilar vein. ×2.

Plate 2

Fig. 1. Radiograph taken during the injection of 0·18 ml. of thorotrast into the upper compartment of the spleen. ×2.

Fig. 2. Radiograph of the same specimen as in fig. 1, 5 min. after injection. ×2.

Fig. 3. Radiograph of the same specimen as in figs. 1 and 2, 10 min. after injection. ×2.

Fig. 4. Radiograph taken during injection of 0·36 ml. thorotrast into the upper compartment of the spleen. ×2.

Fig. 5. Radiograph taken during the injection of 0·12 ml. thorotrast into the middle segment of the spleen. ×2.

Fig. 6. Radiograph taken during the injection of 0·36 ml. thorotrast into the middle segment of the spleen. ×2.

Fig. 7. Radiograph taken during the injection of 0·36 ml. thorotrast into the zone proximal to the lowest segment of the spleen. ×2.

Fig. 8. Radiograph taken during the injection of thorotrast into the lowest segment of the spleen. ×2.

Plate 3

Fig. 1. Radiograph taken during the injection of thorotrast into the upper segment, the upper hilar veins having been previously ligated. ×2.

Fig. 2. Radiograph taken during the injection of thorotrast into the middle segment, the middle hilar vein having been previously ligated. ×2.

Fig. 3. Radiograph taken during the injection of thorotrast into the middle segment, all the hilar veins having been previously ligated. ×2.

Fig. 4. Radiograph taken after injecting thorotrast into the spleen of a dead animal. ×2.

THE DEVELOPMENT OF THE DUCTUS VENOSUS IN MAN AND THE GOAT

By A. D. DICKSON

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The developmental history of the ductus venosus in man may be divided into two stages. The first of these is the establishment of the ductus venosus as the axial vessel of the bilaterally symmetrical arrangement of the major intra-hepatic vessels (Fig. 1). That is to say, the first stage terminates when the ductus venosus, which lies in the mid-line of the dorsal surface of the liver, connects the mid-points of the subhepatic and subdiaphragmatic anastomoses between the right and left omphalo-

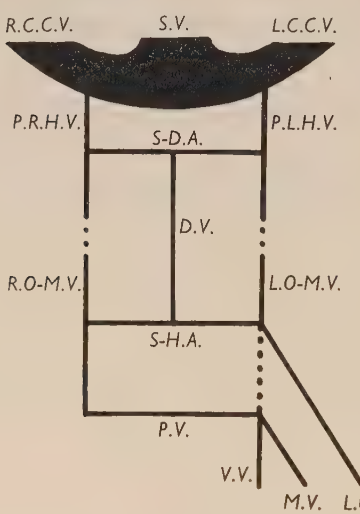

Fig. 1

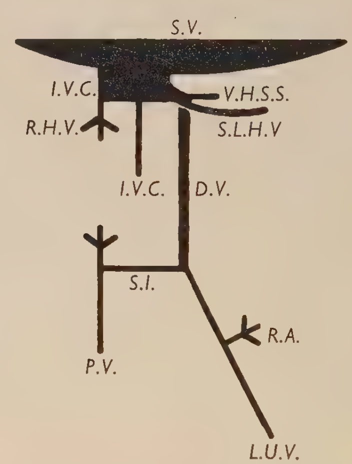

Fig. 2

Fig. 1. The arrangement of the ductus venosus and the related vessels at the symmetrical stage. (For key to lettering, see p. 368.)

Fig. 2. The arrangement of the ductus venosus and the related vessels in the definitive condition.

mesenteric veins. The sequence of the events occurring during this first stage was worked out by Huzly (1942), the symmetrical stage itself having previously been described by Pernkopf (1926). It was, indeed, indicated in Ingalls's (1908) model of the hepatic circulation of a 4·9 mm. human embryo, though he did not appreciate its importance. The second stage of the development of the ductus venosus is that in which the symmetry of the first stage is transformed into the asymmetrical definitive condition of this vessel and of the vessels related to it (Fig. 2). Of the numerous changes which must be involved in this transformation, only two have hitherto been described—the disappearance of the opening into the sinus venosus of the terminal portion of the left omphalo-mesenteric vein (that is, the primary left hepatic vein)

(His, 1885), and the formation of a new, or secondary, left hepatic vein, which runs across the ventral aspect of the cranial end of the ductus venosus to open into the right half of the subdiaphragmatic anastomosis (Schneider, 1937). Since the subhepatic and subdiaphragmatic anastomoses are not apparent in the final arrangement, it is evident that these vessels must be altered in the second stage of the development of the ductus venosus, with which this paper is concerned.

It is necessary to refer to the work of Mall (1906), on which are based the accounts of the development of the ductus venosus to be found in most current text-books of embryology. According to Mall, who did not know of the presence of the subdiaphragmatic anatomosis in the cranial part of the liver, the ductus venosus develops, after the left umbilical vein has been transferred to the hepatic circulation, as an enlargement of the sinusoids lying between this new termination of the left umbilical vein and the common hepatic vein. This has been demonstrated to be incorrect by Huzly (1942), who showed that the development of the ductus venosus has already commenced before the left umbilical vein has begun to be transferred from the sinus venosus to the liver.

The development of the ductus venosus of the goat has not been described previously. As will be shown, it is so similar to that of the known stages of the development of the human ductus that it may legitimately be used to fill in gaps in the developmental history of the human ductus venosus. The vessels of the goat have been given the same names as their human counterparts for the sake of simplicity, though the terms 'subhepatic' and 'subdiaphragmatic' will have slightly different connotations.

MATERIAL AND METHODS

The material utilized consists of 5, 7·75 and 9 mm. human embryos and 6, 7, 8 and 10 mm. goat embryos. In addition, serial sections of thirty-six human embryos and foetuses, ranging in size from 10 to 200 mm. crown-rump length, were examined in order to confirm that the definitive condition has been attained in the 9 mm. human embryo. The 5, 7·75 and 9 mm. human embryos and the 6, 7, 8 and 10 mm. goat embryos were serially sectioned transversely and stained with haematoxylin and eosin. Reconstructions were made of the hepatic vessels of the 5 and 9 mm. human embryos, waxed blotting-paper being used in the first case and wax plates in the second.

OBSERVATIONS

5 mm. human embryo. The ductus venosus, which lies on the dorsal surface of the developing liver, runs cranially in the mid-line from the subhepatic anastomosis (Fig. 3). This anastomosis, which connects the omphalo-mesenteric veins, is a wide channel. The subdiaphragmatic anastomosis, in which the ductus terminates, is comparatively narrow. The right half of the latter anastomosis is larger than the left. Of the other two anastomoses between the vitelline and omphalo-mesenteric veins, the dorsal anastomosis is almost as large as the subhepatic anastomosis, while the caudal ventral anastomosis is incomplete and its remnants are narrower than the dorsal anastomosis.

The left vitelline vein is a channel of considerable size, which runs dorsally and slightly cranially from the umbilicus to the left side of the fore-gut, where it will

receive the mesenteric vein to continue as the left omphalo-mesenteric vein. There is no sign of the right vitelline vein.

The left umbilical vein is very large. It runs cranially and dorsally from the ventral body-wall to join, at approximately a right angle, the left omphalo-mesenteric vein at the level of the subhepatic anastomosis. It gives a branch from its stem in the body-wall to the left lobe of the liver. The right umbilical vein, which is smaller than the left, divides into two. One branch delivers blood into the liver sinusoids of the right lobe, while the other passes dorsally in the body-wall and then courses cranially to join the terminal part of the right omphalo-mesenteric vein (that is, the primary right hepatic vein) just caudal to the sinus venosus.

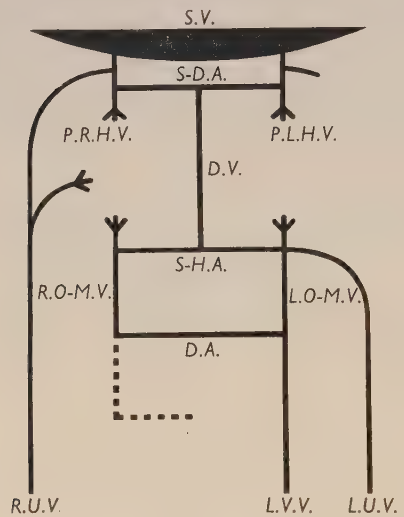

Fig. 3

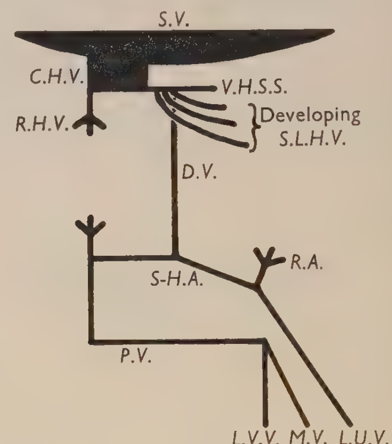

Fig. 4

Fig. 3. The arrangement of the vessels of the 5 mm. human embryo.
Fig. 4. The arrangement of the vessels of the 7·75 mm. human embryo.

The right lobe of the liver is supplied with blood from two sources—the right umbilical vein and the right omphalo-mesenteric vein, the latter receiving its blood from the left vitelline vein through the dorsal anastomosis. The left lobe receives its blood from the left umbilical and left vitelline veins.

The blood which circulates through the liver is collected by the terminal parts of the right and left omphalo-mesenteric veins, each of which has an opening into the sinus venosus. These, the primary right and left hepatic veins, are connected immediately caudal to their openings into the sinus venosus by the subdiaphragmatic anastomosis.

7·75 *mm. human embryo.* The right umbilical vein terminates by joining the left umbilical vein in the ventral body-wall, giving no branches to the liver. The left vitelline vein courses dorsally from the umbilicus to the left side of the duodenal loop of gut, where it is joined by the mesenteric vein (Fig. 4). The vessel formed by this junction, the developing portal vein, passes dorsal to the gut, and, having given off a branch to the right lobe of the liver, terminates in the right end of the subhepatic anastomosis. The left end of this anastomosis receives the termination of the left

umbilical vein and gives origin to the ramus angularis, the main advehent vessel derived from the left omphalo-mesenteric vein and the main vessel of supply to the morphological left lateral lobe of the liver (Rex, 1888). The left half of the subhepatic anastomosis is placed obliquely, the left end being ventral to the right, so that this half now forms an obtuse angle with the umbilical vein, which lies almost in a sagittal plane.

The ductus venosus arises from the middle of the subhepatic anastomosis and runs cranially in the mid-line on the dorsal surface of the liver. The ventral mesogastrium is attached to its left dorso-lateral aspect. The right side of the cranial end of the ductus opens into the common hepatic vein, which almost fills the dorsal extension of the right lobe of the liver.

The drainage of the sinusoids of the dorsal part of the right lobe enters the right side of the common hepatic vein. The blood from the ventral part of the right lobe and from the whole of the left lobe, with the exception of a small dorsal portion, enters the ventral aspect of the common hepatic vein through a series of parallel, inter-communicating channels. On the left ventro-lateral aspect of this vein, there is a wedge-shaped aggregation of trabeculae, which are here more densely packed than elsewhere in the liver. Dorsal to this mass, the sinusoids of the cranial dorsal part of the left lobe drain into the left side of the cranial end of the ductus venosus.

9 *mm. human embryo.* The portal vein passes to the liver in the lesser omentum. Having reached this organ, it is connected (Fig. 5) to the termination of the umbilical vein by a wide anastomotic channel, the portal sinus, or sinus intermedius (Barclay, Franklin & Prichard, 1944). The right lobe is supplied by the portal vein and the left lobe by the umbilical vein. The ramus angularis now arises from the umbilical vein some distance from the termination of the latter.

In this specimen the ductus venosus arises from the left end of the sinus intermedius. It runs cranially in the mid-line at the attachment of the lesser omentum to the liver, and receives, on the left side at the cranial end, a small vein which drains the cranial dorsal part of the left lobe. The ductus venosus opens into the common hepatic vein (which could now be considered to be the inferior vena cava, for there is present a small somewhat plexiform communication with the right subcardinal vein) in common with the vein which constitutes the main drainage of the left lobe. This latter vein, the secondary left hepatic vein of Schneider (1937), passes across the ventral aspect of the cranial end of the ductus venosus to open into the junction of the ductus with the inferior vena cava. This secondary left hepatic vein is joined by the middle hepatic vein, which drains the ventral parts of both lobes. The right hepatic vein, which drains the dorsal part of the right lobe, has a separate opening into the inferior vena cava.

Larger human specimens. Examination of a series of thirty-six embryos and foetuses, ranging from 10 to 200 mm. crown-rump length, shows that the general arrangement of the vessels obtaining in the 9 mm. embryo remains substantially unaltered. The only notable change affecting a major hepatic vessel after the 9 mm. stage concerns the ramus angularis, the origin of which appears to migrate towards the termination of the umbilical vein, reaching it at about the 30 mm. stage.

6 *mm. goat embryo.* The left vitelline vein remains as the sole drainage of the yolk-sac in this specimen, the right vitelline vein having disappeared. The left

vitelline vein and the mesenteric vein open into the left end of the dorsal anastomosis (Fig. 6). This anastomosis thus forms part of the portal vein, the terminal part of which is formed from the right omphalo-mesenteric vein, for this vessel connects the right end of the dorsal anastomosis to the right end of the subhepatic anastomosis, where the portal vein will eventually terminate. The portion of the left omphalo-mesenteric vein connecting the left ends of the dorsal and subhepatic anastomoses is disappearing, for it has lost its opening into the subhepatic anastomosis.

The right umbilical vein opens into the right omphalo-mesenteric vein at the level of the dorsal anastomosis. It is almost as large as the left umbilical vein, which passes dorsally and cranially through the liver to join the left end of the subhepatic anastomosis at approximately a right angle. The subhepatic anastomosis, which lies slightly obliquely, is curved, with the convexity of the curve directed cranially. The ramus angularis takes origin from the left end of this anastomosis, while the

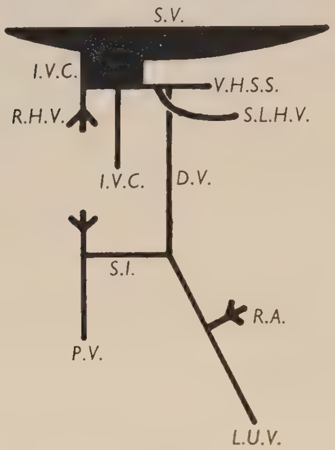

Fig. 5

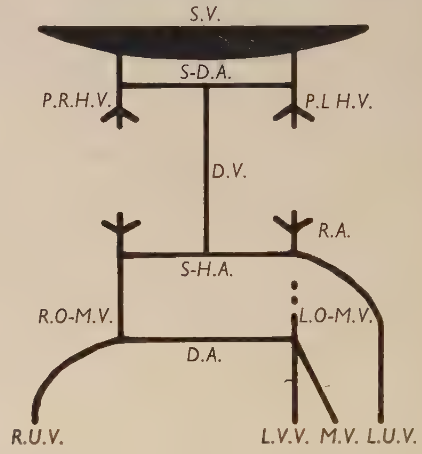

Fig. 6

Fig. 5. The arrangement of the vessels of the 9 mm. human embryo.
Fig. 6. The arrangement of the vessels of the 6 mm. goat embryo.

ductus venosus arises from the summit of its curve. The ductus lies in the mid-line at the attachment of the ventral mesogastrium to the dorsal surface of the liver. It passes cranially and slightly dorsally from its origin, retaining its position in the mid-line. It becomes narrower at its cranial end, which opens into the mid-point of the subdiaphragmatic anastomosis connecting the omphalo-mesenteric veins. The right half of this anastomosis is larger than the left.

The portions of the omphalo-mesenteric veins which lie in the liver have been interrupted by sinusoids. Their revehent parts form the primary right and left hepatic veins, which open separately into the sinus venosus.

7 mm. goat embryo. The right umbilical vein terminates in the left in the ventral abdominal wall, having no longer any direct communication with the liver circulation. The left umbilical vein runs cranially and dorsally on the visceral surface of the liver and terminates in the left end of the sinus intermedius (Fig. 7). The ramus angularis arises close to its termination. The sinus intermedius lies in a transverse plane. It makes an angle of about forty-five degrees with the sagittal plane, the

left end being ventral to the right. The portal vein opens into the right, or dorsal, end of the sinus intermedius, and branches are given off to the right lobe from the junction.

The ductus venosus arises from the left end of the sinus intermedius, and runs cranially, as well as slightly dorsally, in the mid-line at the attachment of the lesser omentum. Into its left side at the cranial end opens a small vessel draining the cranial dorsal part of the left lobe. The ductus is no longer connected with the primary left hepatic vein by the left half of the subdiaphragmatic anastomosis. This connexion has been replaced by the secondary left hepatic vein, which, arising from the primary left hepatic vein, passes across the ventral aspect of the cranial end of the ductus venosus to open, in common with the ductus, into the primary right hepatic vein. The confluence of these vessels constitutes the common hepatic vein, which opens into the sinus venosus. The primary left hepatic vein still retains its separate opening into the sinus venosus. The right half of the subdiaphragmatic anastomosis, which in the 6 mm. goat embryo connected the cranial end of the ductus to the primary right hepatic vein, is no longer a recognizable entity.

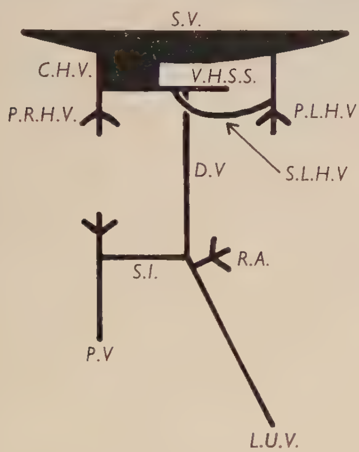

Fig. 7

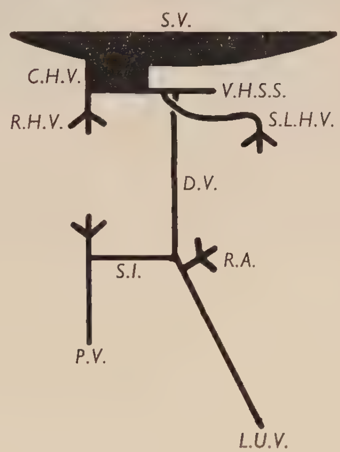

Fig. 8

Fig. 7. The arrangement of the vessels of the 7 mm. goat embryo.
Fig. 8. The arrangement of the vessels of the 8 mm. goat embryo.

8 mm. goat embryo. The opening of the primary left hepatic vein into the sinus venosus has disappeared in this specimen (Fig. 8). The whole of the left lobe, with the exception of the cranial dorsal part, which drains into the cranial end of the ductus venosus, is now drained by the secondary left hepatic vein. This vein passes across the ventral aspect of the cranial end of the ductus, which opens into its dorsal aspect. The common vessel, which is very short, opens into the common hepatic vein, which, in turn, opens into the sinus venosus.

10 mm. goat embryo. A communication has now appeared between the right subcardinal vein and the common hepatic vein. This represents the pre-renal portion of the posterior vena cava (Fig. 9). It is a small vessel, which courses cranially on the dorsal surface of the caudate lobe and then inclines ventrally over the cranial surface to reach the common hepatic vein.

DISCUSSION

It must first be established that the changes occurring during the second stage of the development of the ductus venosus are similar in man and the goat. The ductus venosus, in both the 5 mm. human embryo (Fig. 3) and the 6 mm. goat embryo (Fig. 6), connects the mid-points of the subhepatic and subdiaphragmatic anastomoses between the omphalo-mesenteric veins. Thus at the bilaterally symmetrical stage of the hepatic vessels related to the ductus venosus, the circulatory patterns are similar in the two species. At the definitive stage,* in the 9 mm. human embryo and the 10 mm. goat embryo (Figs. 5, 9), the patterns are also similar, the ductus venosus arising from the left end of the sinus intermedius and terminating in the dorsal aspect of the left hepatic vein, opening into the inferior/posterior vena cava by an extremely short trunk common to these two vessels and to the small vein draining the cranial dorsal part of the left lobe of the liver. Between the symmetrical

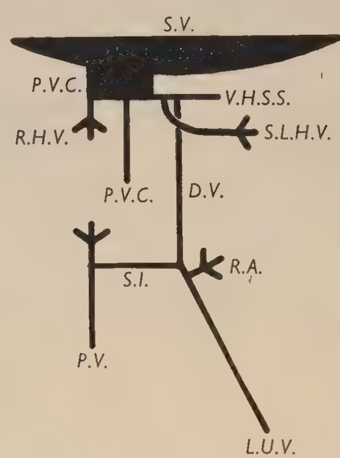

Fig. 9. The arrangement of the vessels of the 10 mm. goat embryo.

and asymmetrical stages, there occurs the disappearance of the subhepatic and subdiaphragmatic anastomoses, as such, in each species. It seems very likely, therefore, in view of these facts, that similar developmental changes occur in man and the goat during this period. It is not suggested that the changes occur in exactly the same order or with the same timing, for the evidence is insufficient to show this. There seems to be no doubt, however, that the changes are similar.

It must be emphasized that the alterations taking place during the second stage of the development of the ductus are alterations of the vessels related to the ends of the ductus, rather than of the ductus itself, for it remains unaltered in position. That is to say, the ductus continues to lie in a sagittal plane, in or near the midline of the body.

* The disposition of the hepatic vessels of the 9 mm. human embryo and the 10 mm. goat embryo is described, for convenience of expression, as the definitive condition of the ductus venosus. This is correct as far as the human ductus is concerned, but it must be noted that the ductus venosus of the goat undergoes further substantial alterations, for it develops a saccular dilatation at its origin, it becomes markedly curved and it is buried inside the liver.

The alterations of the vessels related to the ends of the ductus may be divided into those affecting the vessels related to the origin and those affecting the vessels related to the termination of the ductus. To consider first the alterations of the vessels related to the origin of the ductus from the mid-point of the subhepatic anastomosis, it is evident that the portal sinus, or sinus intermedius, which, in the final arrangement (Fig. 2), lies between the termination of the portal vein and the origin of the ductus venosus, is the right half of the subhepatic anastomosis, since this, in the symmetrical stage (Fig. 1), lay between the origin of the ductus and the advehent portion of the right omphalo-mesenteric vein, which becomes the terminal part of the portal vein. In the definitive arrangement (Fig. 2), the umbilical vein terminates in the left end of the sinus intermedius so that the left half of the subhepatic anastomosis seems to have disappeared. This disappearance may be explained either by the left half of the anastomosis becoming rapidly and completely overshadowed by the growth of the right half, or by the left half changing its position and becoming an integral part of the umbilical vein. The evidence provided by the human embryos is in favour of the latter alternative, because the ramus angularis (i.e. the portion of the left omphalo-mesenteric vein which remains as a vena advehens) originally arose from the left end of the subhepatic anastomosis (Fig. 3), but by the 9 mm. stage (Fig. 5) it has become a branch of the umbilical vein, given off some distance from the termination of this vein. This suggests that the left half of the subhepatic anastomosis has rotated into line with the umbilical vein. Moreover, the 7·75 mm. human embryo illustrates what is apparently an intermediate stage in such a rotation of the left half of the subhepatic anastomosis from the coronal plane into line with the umbilical vein, which lies almost in a sagittal plane (Fig. 4). There seems little doubt, therefore, that in man the left half of the subhepatic anastomosis becomes the terminal part of the umbilical vein.

The fate of the left half of the subhepatic anastomosis is by no means as clear in the goat, for even in the 7 mm. goat embryo (Fig. 7) the ramus angularis arises from the umbilical vein quite close to the termination of the latter. Since, however, it does arise from the umbilical vein and since the distance of its origin from the termination of the umbilical vein is less than the length of the right half of the subhepatic anastomosis (i.e. the sinus intermedius), it seems likely that the left half of the subhepatic anastomosis does rotate into line with, and become part of, the umbilical vein in the goat, though at an earlier stage than in man, and that, furthermore, there is a diminution in the growth rate of the left half of the anastomosis relative to that of the right half, the diminution occurring between the 6 and 7 mm. stages. In man, the origin of the ramus angularis appears to migrate along the umbilical vein, reaching its termination at about the 30 mm. stage, when the origins of the ramus and ductus venosus are quite close together. This would suggest that there is a diminution in the growth rate of what was the left half of the subhepatic anastomosis in man also, but that it takes place at a later stage.

The changes occurring at the cranial end of the ductus venosus are more complex. When the ductus first appears, in man and the goat, the blood in it can pass into both the primary left and right hepatic veins (which are the venae advehentes derived from the left and right omphalo-mesenteric veins), through the corresponding halves of the subdiaphragmatic anastomosis. When the primary left hepatic

vein loses its separate opening into the sinus venosus, all the blood traversing the ductus must now enter the sinus venosus through the primary right hepatic vein, as must also the blood from the left lobe of the liver. Ingalls (1908) believed that the blood from the left lobe returns to the sinus venosus via the left half of the subdiaphragmatic anastomosis, i.e. that the left half of the subdiaphragmatic anastomosis becomes the definitive left hepatic vein. Schneider (1937), however, showed that, in man, a secondary left hepatic vein is formed. The human embryos described above confirm that a secondary left hepatic vein is indeed formed in man, and that it has the course which Schneider described, though they indicate that it develops later than the 6 mm. stage which he suggested, for it is not yet fully formed in the 7·75 mm. embryo. The goat embryos indicate that a secondary left hepatic vein is formed in a similar way in this species, and, further, they illustrate a stage which has not yet been described in man. This stage occurs in the 7 mm. goat embryo (Fig. 7), and shows that the secondary left hepatic vein forms as a vessel which taps the primary left hepatic vein, conveying some of its blood across the ventral aspect of the cranial end of the ductus venosus to the right half of the subdiaphragmatic anastomosis and thence to the common hepatic vein. When the primary left hepatic vein loses its opening into the sinus venosus, all its blood must take this new course. It seems likely that, in the goat, the distal part of the definitive left hepatic vein is derived from the primary left hepatic vein, while the proximal part, with the exception of the terminal portion, which is dealt with later, is derived from the new formation. It is probable that this stage, in which the secondary left hepatic vein taps the primary vein, occurs also in man, and that the left hepatic vein of the adult contains parts derived from the primary and secondary left hepatic veins.

In all the embryos, of both man and the goat, in which the secondary left hepatic vein is developing, or has developed, the drainage of the sinusoids of the cranial dorsal part of the left lobe of the liver is not into the new vessel, but rather into the left side of the cranial end of the ductus venosus. It seems likely that the vein draining this part of the liver is the left half of the subdiaphragmatic anastomosis, in which the direction of the blood flow has been reversed. Ingalls (1908) suggested that the left half of the subdiaphragmatic anastomosis formed the definitive left hepatic vein. Schneider (1937) showed this to be incorrect, and implied that it disappeared. The correct solution would appear to be that it does not disappear, but that it remains to drain a small part of the left lobe. It seems to correspond to the vessel termed, by Elias & Petty (1952) in the adult human liver, the 'venula hepatica cranialis aut superior sinistra'.

The replacement of the primary by the secondary left hepatic vein, and the conversion of the left half of the subdiaphragmatic anastomosis into the vein draining the cranial dorsal part of the left lobe, are not the only alterations affecting the vessels related to the cranial end of the ductus venosus, for the right half of the subdiaphragmatic anastomosis also disappears. The appearances in the specimens described suggest that the channel is almost entirely absorbed into the common hepatic vein, which, by the establishment of a communication with the right subcardinal vein, becomes the inferior/posterior vena cava. It seems likely that, when it first appears, the secondary left hepatic vein opens into the right half of the sub-

diaphragmatic anastomosis close to the termination in the anastomosis of the ductus venosus. Thus, when the right half of the subdiaphragmatic anastomosis is almost completely absorbed into the developing vena cava, the ductus venosus and the left hepatic vein, and also the venula hepatica cranialis aut superior sinistra, open into the vena cava by an extremely short common trunk (e.g. Figs. 5, 9). Since the secondary left hepatic vein passes across the ventral aspect of the ductus venosus, and is, moreover, the larger vessel, the appearance is now of the ductus opening into the dorsal aspect of the left hepatic vein, close to the termination of the latter in the vena cava, with the venula hepatica cranialis aut superior sinistra opening into the angle between the hepatic vein and the ductus. It seems probable that the very short portion of the definitive left hepatic vein, between the entrance of the ductus and the termination in the vena cava, is derived from the right half of the subdiaphragmatic anastomosis. If this is correct, then the definitive left hepatic vein is made up of contributions from three sources, namely, from left to right, the primary left hepatic vein, the secondary left hepatic vein of Schneider (1937) and the right half of the subdiaphragmatic anastomosis. Of these, the second contributes by far the greatest part of the length of the vein.

To turn now to the function of the ductus venosus in relation to its development, it has been suggested (Dickson, 1956) that the function of the ductus venosus is to control the pressure in the sinus intermedius by means of its sphincter, such a controlling mechanism being required because the umbilical and portal veins, in which the pressures may not be at all times in equilibrium, open into the opposite ends of the sinus intermedius. It appears that the development of the ductus venosus, commencing as it does before the transference of the termination of the (left) umbilical vein from the sinus venosus has started, is a preparation for this transference. In other words, the presence of the ductus venosus, with its sphincter mechanism, is necessitated by the termination of the umbilical vein in the hepatic circulation. It is often said that the transference of the umbilical vein occurs because the course of the vein is becoming unduly circuitous with the growth of the embryo. The suggestion is now put forward, tentatively, that the alteration takes place in order that the blood returning laden with nutriment and oxygen from the placenta may come into contact with the cells of the developing liver. That is to say, the need for the transfer may be metabolic and not mechanical. No proof of this suggestion is possible at present, for as yet there is no biochemical evidence that the liver functions at this early stage of embryonic life.

SUMMARY

The alterations taking place during the transformation of the symmetrical arrangement of the ductus venosus of man and the goat are described. It is shown that they are similar in the two species. During the alterations the ductus venosus retains its position in or near the median sagittal plane, for they affect the vessels related to its origin and termination, rather than the ductus itself. The right half of the subhepatic anastomosis becomes the portal sinus, or sinus intermedius, while the left half, it is suggested, becomes an integral part of the umbilical vein. The left half of the subdiaphragmatic anastomosis appears to become the venula hepatica cranialis

aut superior sinistra, draining the cranial dorsal part of the left lobe of the liver, the remainder of this lobe being drained by the definitive left hepatic vein. It is probable that this latter vein is made up of contributions from three sources, namely, the primary left hepatic vein, the secondary left hepatic vein and a small portion of the right half of the subdiaphragmatic anastomosis, the remainder of which is considered to be absorbed into the common hepatic vein prior to the appearance of the pre-renal part of the inferior/posterior vena cava.

I wish to acknowledge my indebtedness to Prof. J. D. Boyd and Dr W. R. M. Morton, who permitted me to study specimens in their collections of human and goat embryos. I am grateful to Prof. J. J. Pritchard for advice and encouragement.

KEY TO LETTERING OF DIAGRAMS

C.H.V.	Common hepatic vein
D.A.	Dorsal anastomosis
D.V.	Ductus venosus
I.V.C.	Inferior vena cava
L.C.C.V.	Left common cardinal vein
L.O-M.V.	Left omphalo-mesenteric vein
L.U.V.	Left umbilical vein
L.V.V.	Left vitelline vein
M.V.	Mesenteric vein
P.L.H.V.	Primary left hepatic vein
P.R.H.V.	Primary right hepatic vein
P.V.	Portal vein
P.V.C.	Posterior vena cava
R.A.	Ramus angularis
R.C.C.V.	Right common cardinal vein
R.H.V.	Right hepatic vein
R.O-M.V.	Right omphalo-mesenteric vein
R.U.V.	Right umbilical vein
S-D.A.	Subdiaphragmatic anastomosis
S-H.A.	Subhepatic anastomosis
S.I.	Sinus intermedius
S.L.H.V.	Secondary left hepatic vein
S.V.	Sinus venosus
V.H.S.S.	Venula hepatica cranialis aut superior sinistra
V.V.	Vitelline vein

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THE OCCURRENCE OF SEX CHROMATIN IN EARLY HUMAN AND MACAQUE EMBRYOS

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INTRODUCTION

It is still uncertain at what stage of human development, and in which tissues, sex chromatin first appears. This paper gives the findings of an investigation carried out on the early human and macaque embryos filed at the Carnegie Institution of Washington (Department of Embryology), Baltimore. Many of these unique specimens are of great embryological interest and have already been described along other lines by Drs Hertig, Heuser, Rock and others. Sex chromatin has been found in the 19-day cat embryo (Graham, 1954), but I have been unable to find reports on embryos younger than this, apart from that of Glenister (1956) who found sex chromatin in the syncytiotrophoblastic cells of an implanting blastocyst, as well as in six out of thirteen embryos whose gonad had not yet differentiated to a testis or an ovary.

MATERIALS AND METHODS

Serial sections (H. & E.) were available of human embryos from the 1-cell stage (Horizon I according to the classification of Streeter, 1942) onwards; and macaque embryos from the free blastocyst stage (Horizon III in the human scale) onwards. Wherever possible, 200 nuclei were examined (2 mm. objective), a procedure often requiring prolonged search since 'readable' nuclei might be widely scattered. Particles were not accepted as sex chromatin unless they lay against the nuclear membrane and possessed the characteristic pyknotic density and plano-convex outline of the sex chromatin body, because this material had not been prepared primarily for cytological studies. The figures for incidence of the sex chromatin may thus be rather low.

The frequency of the sex chromatin within trophoblastic nuclei, however, is even more difficult to assess than in nuclei of the embryo proper because of the nuclear size in trophoblast. For example, a histological section 5μ thick would include the whole of a nucleus measuring $20 \times 5\mu$ only if it lay completely 'flat' within the section, an occurrence which is unlikely. Thus, a large part of a nucleus might be clearly seen in a section, yet its sex chromatin be present in the part of the nucleus outside the section; a false negative reading thus results. To compensate for this difficulty, a method of calculation was used whereby the figure for percentage incidence of sex chromatin obtained by counting the number of particles actually seen in 200 nuclei (or parts of nuclei)—the *apparent incidence*—was corrected so as to produce a figure more nearly approaching the true value. This corrected figure is called here the *calculated incidence*. Details of the method of correction have been given elsewhere (Park, 1957); it consists essentially in multiplying the figure for

apparent incidence by a factor which takes into account the diameter of the nucleus (d) and the thickness of the section (t), both measured in microns. The calculated incidence is the apparent incidence multiplied by $\frac{(2d-4)+t.}{t}$ Although young embryonic cells are more uniform and generally smaller than those of trophoblast, this method of correction was applied to them also. All the figures of incidence mentioned in the text and tables refer to *calculated incidence*.

In trophoblastic syncytium the sex chromatin was sometimes difficult to distinguish because of nuclear pyknosis, although usually it could be seen in both forms of trophoblast with approximately equal ease and frequency. The figures of incidence quoted for trophoblast are the means of the figures obtained for both forms of the tissue or for cytotrophoblast alone.

RESULTS

The total findings for human and macaque embryos are shown in Tables 1 and 2. The specimens (Carnegie Collection numbering) are listed by Horizons, in age order as estimated.

DISCUSSION

So far as present knowledge goes, there seems no theoretical reason why sex chromatin should not be present in the zygote, but in the human and macaque at any rate this appears not to be so. The youngest specimen to show sex chromatin amongst the human embryos was aged 10–12 days, the youngest macaque was aged 10 days. In both, the sex chromatin was seen in the trophoblast only, and in neither did the incidence exceed 3 %. These were the only two specimens, out of the eighteen 'readable' specimens aged up to 12 days, that showed any sex chromatin. Since it is unlikely that all eighteen were male the virtual failure to find sex chromatin may be taken as showing it to be normally absent up to this age. In younger specimens it is not always easy to be sure that cells containing sex chromatin at the junctional zone are not maternal cells. As may be gathered from Table 1, no sex chromatin was seen in the large, certainly trophoblastic cells in this zone in specimens of Horizons III and IV; but it was seen in many of the smaller cells lying amongst them. These small, mature nuclei, which, as pointed out by Hertig & Rock (1945), tend to congregate near the maternal tissue, were thought by these authors to be formed by amitotic division of the original nuclear mass of syncytium. The presence of sex chromatin within some of them (and we are almost certainly both referring to the same nuclei) makes it seem likely that these nuclei at any rate belong to maternal stromal cells blended with the developing trophoblast.

The first appearance of the sex chromatin is in the trophoblast and chorionic mesoderm. Although, as the tables show, this is not absolute, there seems to an earlier rise there than in the embryo itself and it is difficult to believe that this could be due to any artifacts of histological technique. This differential first appearance would seem to support the validity of the finding that at an earlier stage there are in fact no sex chromatin particles.

In the human embryo, sex chromatin particles in significant number were seen first at the eighteenth day, in the macaque at the nineteenth. The sixteen specimens,

(eleven human, five macaque) from the eighteenth day onwards can be divided into two main groups: six cases where some part of the embryo or its trophoblast showed an incidence of sex chromatin of 40 % or more, suggesting female tissue, and ten cases with a corresponding incidence of 13 % or less, suggesting male tissue (in four of these latter no sex chromatin was seen in any area). It seems, therefore, that a definite sex difference exists certainly by the eighteenth day in both species and, on

Table 1. *The distribution of sex chromatin in embryonic tissue of the human*

Specimen number and stage of development	Percentage incidence of sex chromatin in: Embryo	Trophoblast	Chorionic mesoderm
Horizons I–IV (up to 9 days)			
8698* (2-cell egg, 36 hr.); 8450* (abnormal 8-cell morula, 48–72 hr.); 8452* (abnormal 12-cell morula, 72 hr.); 8155† (8 days); 8215 (8 days); 8225 (8 days); 8171† (9 days)	Nil	Nil	Nil
Horizon V (10 to 12 days)			
7770; 7950; 8558	Nil	Nil	Nil
8000	Nil	<1	Nil
Horizon VI (13 to 14 days)			
7801‡	Nil	16	10
8290	Nil	Nil	Nil
8602	<1	9	24
Horizon VII (about 16 days)			
7762	12 (yolk sac)	Nil	10
7802‡	<1 (yolk sac)	Nil	Nil
8752	Nil	Nil	Nil
Horizon VIII (about 18 days)			
7666	21 (amnion) 63 (notochord)	37	30
7701	26 (amnion) 45 (yolk sac)	22	25
7949; 8727	Nil	Nil	Nil
Horizon IX (about 20 days)			
7650	31 (body wall) 6 (neural plate)	20	43
Horizon X (about 22 days)			
2795	Nil	<1	Nil
3710	Nil	Nil	Nil
5074	<1	6	10
Horizon XI (about 24 days)			
7611	2 (neural tube)	Nil	Nil
7851	77 (neural tube)	40	80
8005	<1	Nil	<1

* Hertig *et al.* 1954. † Hertig & Rock, 1949. ‡ Heuser *et al.* 1945.

the findings in the human Horizon VI specimens, possibly by about the fourteenth day, although such sex chromatin as was seen at this stage was present almost exclusively in the trophoblast, not in the embryo itself. Incidentally the sexing of early embryos has depended till recently on recognition of changes within the gonad, for example the sex cords of the male gonad, appearing at about the fortieth day,

and thus this present work confirms the suggestions of Graham (1954) and Glenister (1956) that identification of sex chromatin would prove a way of sexing embryos before the fortieth day.

Table 2. *The distribution of sex chromatin in embryonic tissues of macacus rhesus*

Specimen number and stage of development	Percentage incidence of sex chromatin in		
	Embryo	Trophoblast	Chorionic mesoderm
9 days			
C560	Nil	Nil	—
C610	Nil	Nil	—
10 days			
C548	Nil	Nil	—
C532	Nil	Nil	—
C496	Nil	1 in unattached portion. 3 in 'deep' portion	—
11 days			
C599	Nil	Nil	—
C524	Nil	Nil	—
13 days			
C595	Nil	<1	—
C466	Nil	1	—
C562	Nil	Nil	—
C467	Nil	1 in unattached portion. 4 in 'deep' portion	—
15 days			
C571	Nil	Nil	Nil
17 days			
C457	Nil (germ disk) (2) amnion	12	2
18 days			
C546	Nil	Nil	Nil
19 days			
C508	66	43	66
20 days			
C421	12	13	7
29 days			
C477	6	Nil	Nil
34 days			
C479	54 (neural tube) 80 (paraxial mesoderm)	22	16

It may be concluded that in the human and macaque embryo, the period of absent sex chromatin is followed by a gradual appearing of these bodies from about the fourteenth day, predominantly at first in the trophoblast, and then in the embryo proper. By about the eighteenth day there are enough to indicate that the sex difference has been established. As soon as the incidence of sex chromatin in the embryo proper is enough to allow a reasonable comparison with the trophoblastic zone, the possibility arises of determining whether the sex of the trophoblast and that of the embryo is always the same. The findings in this material have shown that they are always identical.

Finally, the study of embryos during the period when the sex chromatin is in process of appearing has revealed an unusual variation from place to place in the rate of appearance. The present material is inadequate in amount and had not been processed by techniques ideal for study of the sex chromatin, and it is therefore not possible even to discern a pattern of sequence. The interesting possibility remains,

however, that different tissues respond differently and perhaps sequentially to the stimulus that begins to call forth, from about the twelfth day, the sex chromatin.

SUMMARY

1. A series of thirty-three human and eighteen macaque embryos has been examined in an attempt to assess the time of appearance and distribution of sex chromatin at early stages of development. The human embryos were aged from approximately 36 hr. (a two-cell egg) to 24 days, and the macaque embryos from 9 to 34 days.

2. In the human, sex chromatin was first seen at the age of approximately 12 days, in the trophoblast; and in the embryo itself at approximately 16 days. In the macaque, it was seen very occasionally from the tenth day onwards in the trophoblast, and in greater number in the embryo itself at the nineteenth day.

Other specimens of equal age showed no sex chromatin in any area, suggesting that a reliable sex difference is established in both species between about the twelfth and nineteenth days.

3. In the older specimens, sex chromatin was always present in the trophoblast of embryos showing sex chromatin, and absent from the trophoblast of embryos not showing sex chromatin.

4. The present study suggests that sex chromatin appears at different times or with different rates in the different tissues of the embryo.

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PLACENTATION IN THE SPOTTED HYENA (*CROCUTA CROCUTA* ERXLEBEN)

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The death of a multiparous spotted hyena in the second stage of labour presented an opportunity for the examination of the full-term placenta and membranes of this interesting animal. Matthews (1954) briefly records some morphological and histological data, and establishes that the placenta is haemochorial and not endotheliochorial in type as are all other known carnivore placentae. Amoroso (1955) also briefly comments on the placenta and shows an excellent coloured photomicrograph of the labyrinthine strands. An account of the full-term placenta and membranes is now given, but until specimens are available from all stages of pregnancy the full story cannot be written.

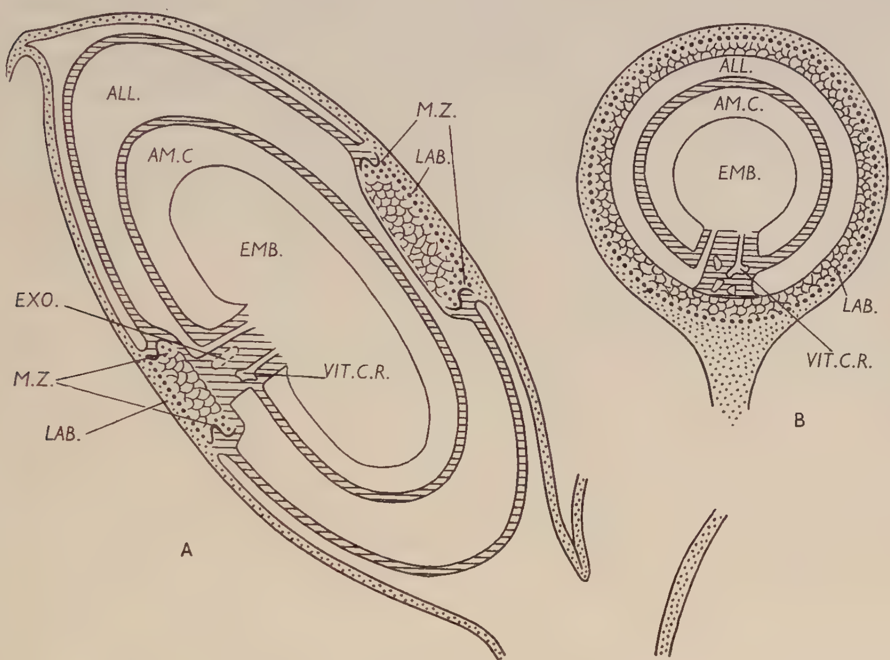
MATERNAL HISTORY

A mature female, about 10 years of age at death, had littered successfully on three previous occasions since the age of 4 years, giving birth to two, three and one young in the order of pregnancies. A fourth pregnancy terminated on 3 January 1956, with the sudden collapse and death of the animal. The intact body was slowly perfused with a formol-dettol mixture through the right common carotid artery. On examination a female pup was found to be inextricably wedged in the maternal pelvic outlet. The membranes of this, and of a male pup, both from the right uterine horn, were detached and partly macerated. An undisturbed chorionic sac containing a second male foetus was present in the left uterine horn, and served as the basis of the present description.

GROSS ANATOMY OF THE MEMBRANES AND PLACENTA (Text-fig. 1)

The male foetus of 27 cm. C.R. length and of a uniform almost black colour lay vertex down in the left horn of the uterus, with its spine dorso-laterally in the curve of the unattached border. It was enclosed in an amnion which contained about 500 ml. of clear yellowish fluid unsoiled by meconium. The amnion was fused to the 6 cm. long umbilical cord and to the allantoic vesicle; this last named lined the chorion and contained rather more fluid than did the amniotic sac, and its duct was still patent within the umbilical cord. The remains of the thin-walled yolk-sac were found between the amnion and allantois, and its stalk with the vitelline vessels ran parallel to, and almost detached from, the allantoic stalk although both were enclosed in a common amniotic sheath (Pl. 1, fig. 4). The dilated vitelline vein crossed the upper abdomen as a very fine thread to end in the pancreatico-duodenal region, while the vitelline duct and artery continued across the general peritoneal cavity in a firm white cord which joined the left leaf of the mesentery of the small gut (Pl. 1, fig. 3). Two umbilical arteries and two umbilical veins ran straight courses through the cord, the mesoderm of which was well supplied by fine blood vessels which also

acted as vasa vasorum for the main vessels. Traced towards the placenta (Pl. 1, fig. 2) one umbilical artery supplied the upper and the other the lower half of the annular band by branches which ran to right and left. Each umbilical vein drained unequal territories on the right and left halves of the placenta. Communicating veins were present at the abumbilical region between the veins of the right and left halves and, more proximally, between the veins of the upper and lower zones. Free venous drainage of all parts of the placenta was thus assured. The placenta, which formed a complete annulus around the central part of the ovoid chorion, was 40 cm. in circumference and varied in width from 11 cm. antimesometrially, to 3·5 cm. mesometrially near the attachment of the umbilical cord, and in thickness from 1·4 cm. at the lower edge to 0·9 cm. at the tubal edge. Its colour varied from dark red to purple-blue on the foetal aspect, with no marginal colour change. When detached from the uterus its maternal side was seen to be greyish yellow in colour, and over one hundred conspicuous venous openings protruded externally (Pl. 1, fig. 1). The openings had been produced by a tearing away of maternal placental veins from an extensive retro-placental venous network, which in places remained attached to the placental surface of the uterus, or the uterine surface of the placenta. The ovaries contained recent and older generations of corpora lutea (Pl. 1, fig. 5) as in the manner described by Matthews (1939). Some small follicles with antra were also present.



Text-fig. 1. Diagrams to illustrate the relations of the foetal membranes in (A) longitudinal section, and (B) transverse section of a uterine horn. *ALL.* allantoic cavity; *AM.C.* amniotic cavity; *EMB.* embryo; *EXO.* exocoelom; *LAB.* labyrinthine part of the placental annulus; *M.Z.* marginal zones with cytotrophoblastic fronds and maternal symplasma; *VIT.C.R.* vitelline cavity remnant.

MICROSCOPICAL TECHNIQUES

A portion of the widest part of the placenta with its adjacent chorion and the overlying uterine wall was bisected in the long axis of the placenta. Both halves of the placenta, with the attached and undisturbed uterine wall, were serially sectioned transversely to this long axis and at right angles to the uterine wall, and a block from one half was also sectioned through its full thickness in a direction tangential to the placenta and uterine wall. Sections, cut at 4, 6 or 10 μ, were mounted serially, and the majority were stained by haematoxylin (Harris) and eosin. Some sections were stained by Weigert's iron haematoxylin and van Gieson, the triple connective tissue stains of Mallory and Masson, the periodic acid-Schiff (with controls after diastase digestion), and Wilder's silver reticulin impregnation methods. The presence of iron was tested for by the prussian blue and the Turnbull blue methods and by micro-incineration, and of calcium by von Kossa's test and by alizarin staining. Frozen sections were tested for fat by staining with Herxheimer's solution (scarlach R and sudan IV).

MICROSCOPICAL APPEARANCES

Sections cut transversely to the long axis of the placenta show (Pl. 1, figs. 6, 7), in succession from the foetal to the maternal aspect, the following layers: (1) a thin allantoic wall thrown into occasional shallow folds and lined by columnar or cuboidal epithelium; (2) chorionic mesoderm from which numerous strands project into the placental mass; (3) the placental mass proper, of fine chorionic mesodermal strands covered by very thin syncytiotrophoblast in which are numerous foetal capillaries; (4) the apices of the placental folds or strands attached by syncytio- or cyto-trophoblast to a thin junctional layer or basal plate composed of altered blood, glandular debris, dilated uterine glands and maternal giant cells; (5) a cleavage zone of loose areolar tissue; (6) a narrow basal glandular zone containing the ends of a few scattered uterine glands, which are separated by a little areolar tissue from (7) the uterine muscle layer and peritoneum.

In the main placental mass the syncytial-covered mesodermal strands form lamellae about 0·2 mm. thick (Pl. 1, figs. 8, 9) which run obliquely outwards from the chorionic plate to the basal plate, and, anastomosing with neighbouring lamellae, form a honeycomb-like labyrinth some 8–10 mm. in thickness (depth). The lamellae are relatively close together and the interlamellar spaces contain maternal blood. Free-ending processes are also seen and appear to represent sections of the edges of lamellae and not of true villi (Pl. 2, fig. 12). In sections tangential to the chorionic plate (Pl. 1, fig. 9) the labyrinthine architecture is most readily understood. Large foetal vessels are situated at the axes of radiating mesodermal strand systems, all of which are covered by syncytiotrophoblast. These vessels run at right angles to the chorionic plate, and give off smaller vessels which supply, or drain, capillaries in the lamellae. These capillaries are very numerous and are best seen in transverse sections where they are cut across. Many of the capillaries lie very superficially in the lamellae and are surrounded by syncytium which is greatly attenuated over their protruding outer walls (Pl. 2, figs. 10, 11). Other capillaries are more deeply placed and lie in the mesoderm of the lamellae. The syncytiotrophoblast varies considerably

in thickness from the extremely attenuated superficial layer covering capillaries, to large masses present here and there along, and at the free margins of, lamellae (Pl. 2, fig. 12). The syncytial nuclei stain intensely and are either small ($0{\cdot}0063 \times 0{\cdot}0037$ mm. in diameter) or greatly elongated, irregularly lobulated structures. Numerous granules, which stain yellow with H. and E., and deep black with von Kossa's reagent, are present within the thickness of the lamellae. They appear to be deposits of denatured blood pigment, which possibly have become calcified. The lamellae at the foetal-maternal junctional zone are covered either by tall columnar cytotrophoblast or by a syncytiotrophoblast, and are attached to a thin layer of symplasma composed of degenerated maternal blood, glandular debris and dilated uterine glands, the lumina of which are filled with cellular debris. Large sinusoidal venous channels in the junctional zone of the placenta drain directly into the retro-placental veins previously mentioned. Small thick-walled maternal arteries (Pl. 1, figs. 6, 7) can be seen crossing the cleavage zone obliquely and opening into the labyrinthine spaces at the junctional zone. Giant cells, with conspicuous large single or multiple nuclei, and poorly staining cytoplasm, lie in the deeper part of the junctional zone, and may occasionally form part of the maternal wall of the placental blood space. They vary greatly in number from one region to another, and stain less deeply than syncytiotrophoblast. They appear to be of maternal origin, some coming possibly from degenerated gland ducts, the cells of which have lost their boundaries, and coalesced.

The marginal regions of the placenta show extensive proliferations of the cytotrophoblast (Pl. 2, figs. 13, 14). The cells are arranged in large plicated fronds or arborizations, and are taller than the cells covering the apices of the lamellae in the junctional zone. These fronds project towards the interior of the placental band, undercutting the main mass of the labyrinth from the side, and separated from it by a considerable mass of degenerated blood and glandular debris similar to that of the junctional layer. The nuclei of the columnar cells in the fronds are large, may be basally, centrally or apically situated, and have conspicuous nucleoli. The cells rest on a basement membrane which intervenes between them and the almost cell-free mesodermal core. This mesoderm is strongly basophilic in some places, and both here and in the junctional zone shows a marked reaction for iron with the prussian blue, Turnbull blue and micro-incineration methods. Calcium, as shown by von Kossa's method and by alizarin staining, is also present in this basophilic mesoderm, but the reactions are not nearly so strong as those for iron. Ingested maternal red blood cells can be identified in many of the columnar cells (Pl. 2, fig. 16), and, in frozen sections stained with Herxheimer's reagent, sudanophil droplets, which become progressively smaller as the bases of the cells are approached, are very numerous. Fuchsinophil droplets, following Mallory's stain, are also conspicuous in these cells. The surrounding symplasma is rich in sudanophil droplets, but in the labyrinthine lamellae they are fewer in number, smaller in size, and confined to the syncytiotrophoblast. No colour change was noted macroscopically at the margins of the placenta, but in the stained preparations there is a distinct tinctorial difference between symplasma here and at the junctional zone, and the blood in the main placental labyrinth. The marked reactions for iron and fat suggest that the marginal and junctional regions are functionally equivalent to the 'green border' in the placenta of the dog.

The paraplacental zone of the chorion is thrown into low folds which interdigitate with similar folds of the uterine lining. The single layer of columnar cells covering the trophoblast in this and other non-placental areas is generally similar to that covering the fronds, except that the cells are not so tall, and in places have distal vesicular portions like those described by Ewart (1915) in the trophoblast of the horse. Maternal red blood cells and sudanophil and fuchsinophil inclusions are also present in these cells.

The underlying chorionic mesoderm in some areas is highly basophilic and with strong positive reactions for iron, as in the marginal and junctional areas of the placenta. The paraplacental uterine wall is covered by low columnar or cuboidal epithelium which strips easily from the loose connective tissue beneath. Uterine glands are small, and not a conspicuous feature either here or in the placental zone. Glycogen was not detected in any part of the placenta or uterine glands by the P.A.S. method controlled by diastase digestion. This result was to be expected as formol-dettol fixation does not preserve glycogen.

DISCUSSION

The foetal membranes, in general, show gross characters similar to those of other Carnivora. Thus, the placenta forms a complete zonary band around the equator of the chorion, the allantois is large and lines the chorion, the yolk-sac remnant, though small, is recognizable at full-term, and the vitelline vessels persist in their intra-abdominal course at full-term. The most arresting difference lies in the haemochorial nature of the placental labyrinth, for no other known carnivore exhibits this arrangement. Conditions for foetal-maternal exchange must, therefore, differ considerably from those in other carnivore placentae. The maternal septa penetrating to the chorionic plate with stem arteries, as in the cat (Duval, 1895) and the dog (Duval, 1894), are absent. Instead, as in the monkey (Ramsey, 1956), the maternal blood is discharged through the basal plate into a labyrinthine blood-lake bathing the chorionic lamellae, and is drained through the basal plate again via sinusoidal openings into obliquely placed veins, which form a retro-placental network. No marginal sinus, such as is claimed by Spanner (1935) to be present in the haemochorial placenta of the human, exists in the hyena placenta.

The orientation of the main foetal blood vessels within the chorionic lamellar systems is similar to that of other Carnivora, with stem vessels proceeding directly towards the basal plate, giving off smaller vessels as they go, so that the capillary blood flow is probably directed back towards the chorionic plate. Intrasyncytial capillaries are extremely numerous and widely distributed throughout the placental labyrinth. A gradation in the numbers of such intra-trophoblastic capillaries is stated to exist among the endothelio-chorial carnivore placentae from the timber wolf up to a climax in the grey Atlantic seal (Amoroso, 1955). Intra-trophoblastic capillaries are also found in *Perameles* (Hill, 1897) and in many Ungulata.

The foetal labyrinth does not show the lobulation, or the relatively unbranched lamellae, of the cat; but, like the cat, the lamellae at the junctional zone tend to be capped with cytotrophoblast. This cytotrophoblast, and that of the more conspicuous marginal fronds, closely resembles the cytotrophoblast of the 'haematomal areas' of other Carnivora, but the cells are taller than those in the full-term dog,

shorter than in the full-term cat, and of about the same height as in the 97 mm. C.R. badger placenta (personal observation). They contain ingested and partially destroyed maternal red blood cells, in this respect most closely resembling the condition seen in the cat. Sudanophil droplets are larger and more numerous in these cells than in other parts of the placenta. In this connexion Wislocki & Dempsey (1946) showed that the syncytiotrophoblast of the labyrinth, and to a lesser degree the cytotrophoblast flanking the junctional zone in the cat placenta, was rich in fat droplets.

The low columnar cytotrophoblast cells in the paraplacental and non-placental areas in the hyena also contain ingested maternal red blood cells and sudanophil droplets, and the underlying stroma, in places, reacts strongly for iron. The ability to engulf particulate matter is said by Dempsey (1955) and Amoroso (1955) to be a characteristic of the membranous chorion in the Carnivora, and Amoroso (1952) reported paraplacental rosettes in dogs, which, although no cellular debris is seen within the cells, are thought to be for absorption. Bonnet (1882) showed that the ruminant trophoblast is actively phagocytic, and Jenkinson (1906) found ingested maternal corpuscles and cellular debris in the inter-cotyledonary columnar trophoblast in the cow and the sheep, while Björkman (1954) demonstrated a wide distribution of sudanophil granules in those areas of the bovine chorion. The presence of maternal corpuscles and fat granules within the non-placental cytotrophoblast of the hyena is not therefore without precedent, but the wide distribution and lack of specialized arrangement of the ingesting cells is of interest.

It is apparent from a comparative study of the endotheliochorial carnivore placentae that they can be graded according to the complexity of the labyrinth, the size, situation and number of the haematomata, the development of the maternal labyrinthine capillaries and amount of surrounding interstitial matrix or basement membrane, the amount of the maternal glandular tissue deep to the placenta, or by almost any feature of the placenta. For example, the labyrinth is regular and lobulated in the cat, irregular and not lobulated in the seal; maternal capillaries are large sinusoidal vessels lined by prominent cuboidal epithelium and surrounded by a thick amorphous matrix in the badger, but are much smaller and more like ordinary capillaries in the cat and the dog, where they are lined by a lower type of endothelial cell and have less supporting matrix externally; large marginal haematomata occur in the dog, central and marginal ones occur irregularly in the cat, central ones in the badger and seal, and some carnivores are said (Amoroso, 1952) not to have a haematoma. Giant cells, which in the hyena placenta are large and resemble those of the horse (Matthews, 1954), are present only in the cat among the other carnivores (Wislocki & Dempsey, 1946). The amount of maternal glandular reaction in the hyena is small, and the virtual absence of a spongy glandular zone, either at the junction of foetal and maternal tissues or in the basal glandular region, contrasts markedly with the conditions found in the cat, dog and sea-lion. It is evident that the reaction of the maternal tissues to the invading trophoblast, and the amount of maternal blood extravasated, differ among the Carnivora. In the hyena the early invasion of the trophoblast must outstrip the proliferative reaction of the uterus, and so cause the maternal blood vessels to become eroded over a wide area. A large generalized blood-lake, in which maternal blood circulates, is thus established

instead of regional haematomata in which circulation does not occur, as in other Carnivora.

An examination of the placentae of a full series of developing hyena embryos would be necessary to confirm this suggestion, but evidence so far presented does suggest that the haemochorial condition could have arisen by an extension of the maternal extravasations associated with a suppression of maternal capillary growth.

SUMMARY

1. An account of the gross morphological, histological and histochemical appearances of the placenta in a full-term spotted hyena is given.

2. The placenta forms a complete zonary band, or annulus, around the equator of the chorion, the allantois is large and lines the chorion, and the yolk-sac remnant can be recognized between the allantois and the smaller amnion.

3. No coloured border is present at the margins of the placenta, but specialized cytotrophoblast there, and at the junctional zone, contains maternal red blood corpuscles, and sudanophil droplets presumably derived from the surrounding symplasma of extravasated blood and glandular debris.

4. The paraplacental and general chorionic cytotrophoblast also contain ingested maternal red blood cells.

5. Iron is present in some of the mesoderm deep to the specialized cytotrophoblast of the placenta and to some parts of the general chorionic cytotrophoblast.

6. Maternal giant cells, which may be extremely large multinucleated structures, are present in the junctional zone.

7. Uterine glands are inconspicuous in all regions of the uterus.

8. A comparison of the main features of the hyena placenta is made with similar features in other Carnivora.

9. It is suggested that a graded series can be made within the Carnivora which show degrees of maternal reaction to the trophoblast.

10. It is suggested that the haemochorial placenta of the hyena is developed as an extreme form of haematoma formation with an associated suppression of maternal capillary growth.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. The maternal aspect of the placenta. Large venous openings which drain maternal blood from the interlamellar labyrinth can be seen. ×0·9 approx.

Fig. 2. Foetal aspect of the right half of the placenta showing the large marginally placed vessels joining it to the umbilical cord. ×$\frac{1}{2}$ approx.

Fig. 3. View of the intra-abdominal part of the vitello-intestinal stalk (*Vit.s.*) in the pup, as it runs from the umbilicus to the left side of the mesentery of the small intestine. The patulous opening of the urogenital orifice (*U.G.O.*) can be seen below the attachment of the umbilical cord. ×$\frac{2}{3}$.

Fig. 4. Transverse section of the umbilical cord with the attached vitelline stalk (*Vit.s.*). The two umbilical veins are above and the allantoic duct (*All.*) lies between the two arteries below. Numerous small blood vessels are scattered throughout the mesoderm of the cord. The vitelline stalk here contains a single prominent vein, numerous small arteries and the remnant of the vitello-intestinal duct. ×4·4.

Fig. 5. Longitudinal section of the right ovary showing a large recent corpus luteum (*C.L.*), several older corpora lutea, and part of the very vascular hilum. ×3.

Figs. 6, 7. Two transverse sections, which together represent a complete transverse section of the placenta. Large allantoic vessels are above the main placental mass, and two small maternal arteries (*M.art.*) cross the cleavage space obliquely from the uterine wall to the thin, darkly staining junctional zone. ×1·2.

Fig. 8. Transverse section of the middle region of the main placental labyrinth. The interlamellar spaces are filled with maternal blood. ×30.

Fig. 9. Tangential section of the middle region of the labyrinth. Two large foetal veins (*V.*) and one artery (*A.*), cut transversely, are lying in mesodermal cores at the axes of radiating lamellae. ×30.

PLATE 2

Figs. 10, 11. Transverse section of the foetal labyrinth. Numerous foetal capillaries (*Cap.*) are partially or completely surrounded by syncytiotrophoblast. A blood-filled foetal capillary is also present in the mesoderm of the right upper strand. The branching nature of the chorionic strands is readily apparent. ×567.

Fig. 12. Transverse section of a free-ending labyrinthine lamella with capillaries surrounded by a syncytial mass. ×567.

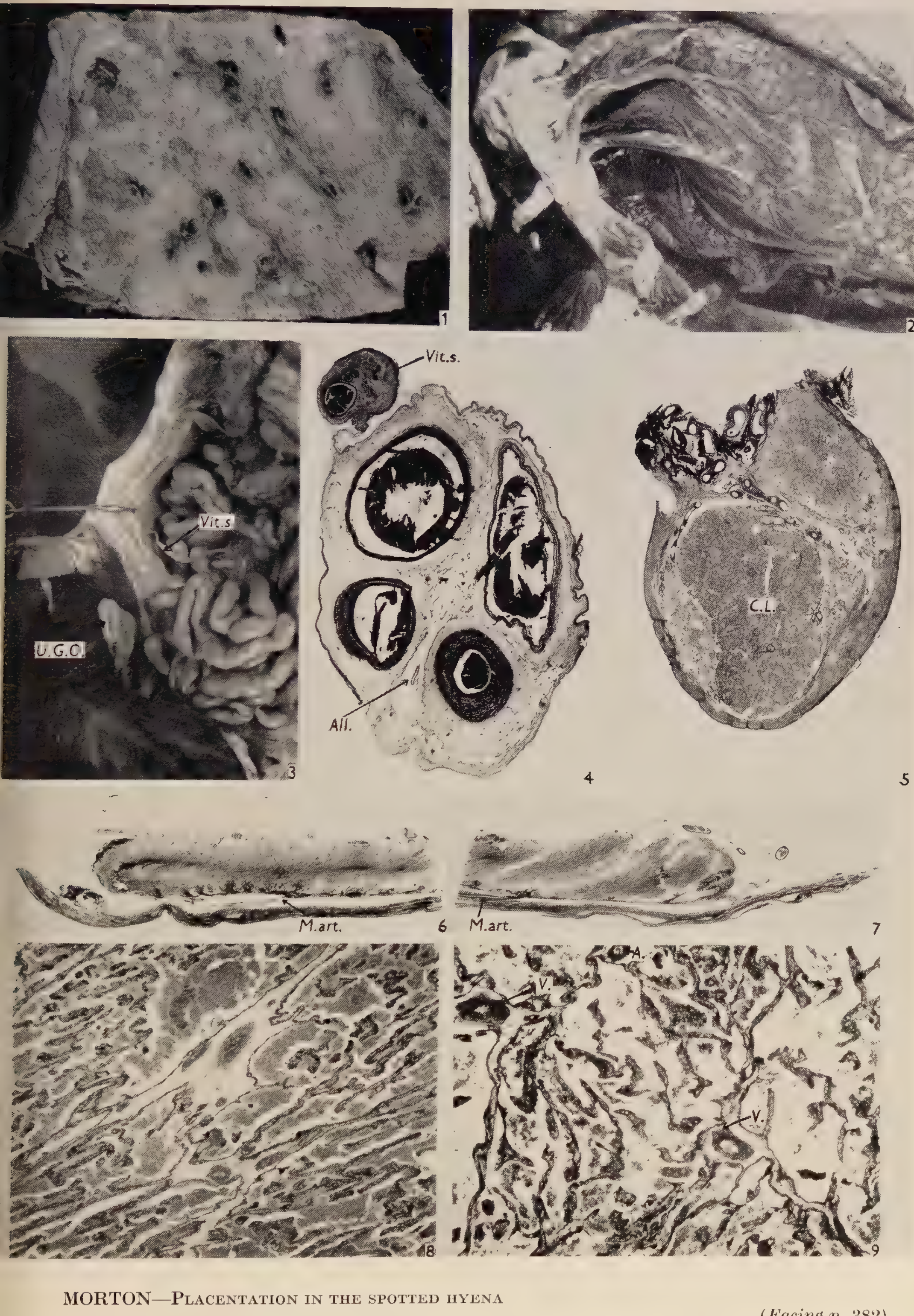
Fig. 13. Transverse section of part of the marginal arborization of the cytotrophoblast. The basophilic mesoderm underlying the columnar cells appears as a dark line, and the amorphous symplasma indents the folds from the right. ×57·4.

Fig. 14. Transverse section of the right marginal zone. Cytotrophoblast fronds project into a symplasma, consisting of altered maternal red cells, glandular debris and amorphous degenerative products, and continue into the junctional zone below. Darkly staining basophilic mesodermal (*B.M.*) cores of these folds are continuous with the looser allantoic mesoderm on the right. Dilated uterine glands (*GI.*) lie just above the uterine muscle. $\times 9$.

Fig. 15. A 10μ thick section from the right marginal zone photographed by reflected light after micro-incineration. The granules of yellow iron oxide from the mesoderm of the marginal folds show up as a broken white line (*I.O.*). (Compare with fig. 14.) $\times 9{\cdot}2$.

Fig. 16. H.P. view of columnar cytotrophoblast from a marginal fold. The cells have a granular or vacuolated cytoplasm and contain ingested maternal red cells (*R.B.C.*) at their apices. $\times 936$.

Fig. 17. Transverse section of junctional zone. Four multinucleate giant cells (*Gi.c.*) lie in the symplasma below a placental blood space. A downward projecting cytotrophoblast frond is on the right. $\times 156$.



MORTON—Placentation in the spotted hyena

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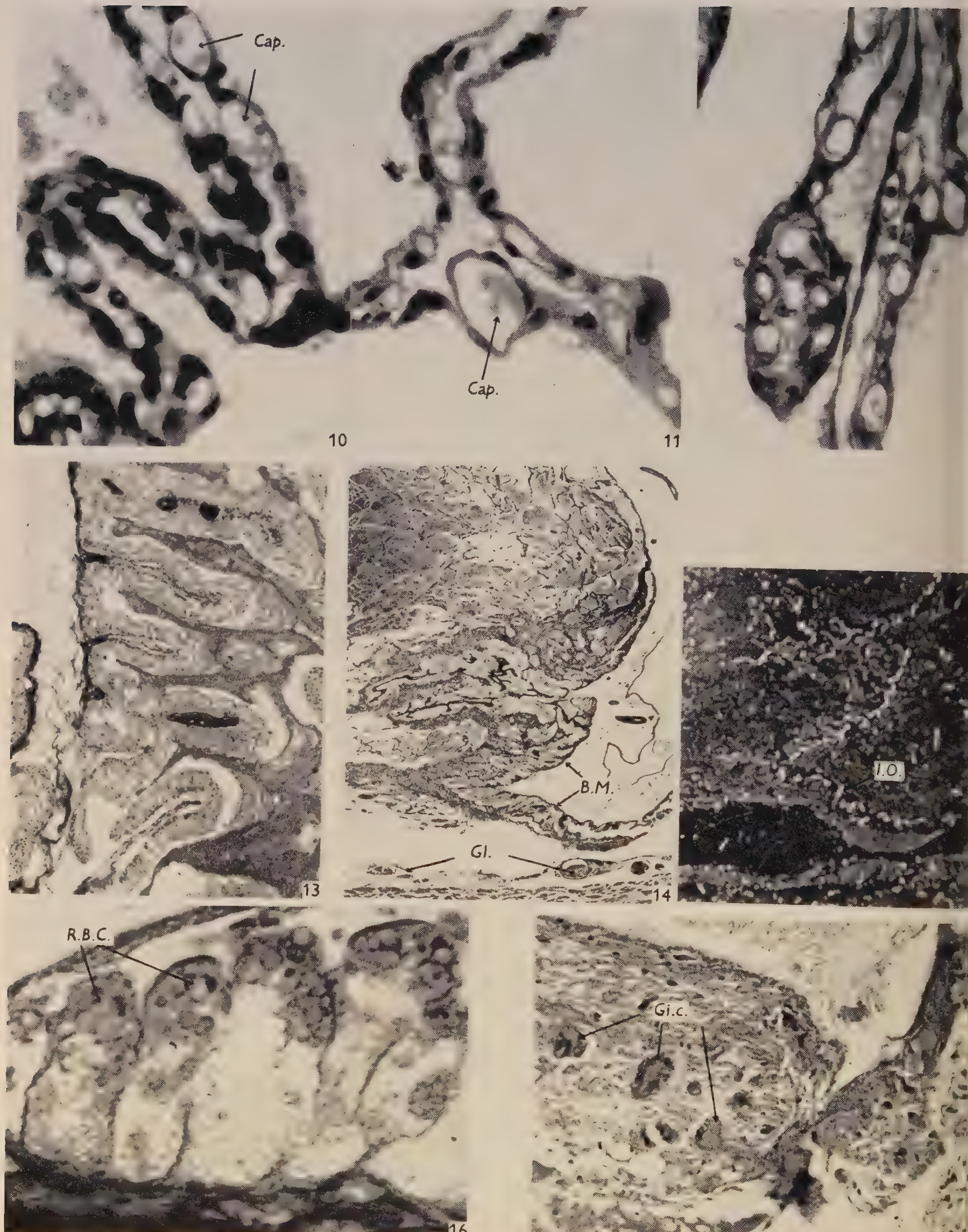

MORTON—Placentation in the spotted hyena

THE CORRELATION OF STRUCTURE AND FUNCTION IN THE MESONEPHROS AND METANEPHROS OF THE RABBIT

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INTRODUCTION

The problem of the function of the mesonephros has been studied by many workers, notably Gersh (1937), who used intra-vitam injections of ferrocyanide and phenol red, and concluded that in certain forms both the glomeruli and tubules could function, and that degeneration of the mesonephros in them is not related to the structure of the allantois or placenta. Earlier, Bremer (1916) investigated the histological inter-relations of the mesonephros, kidney and placenta and concluded that in the rabbit urinary excretion in the embryo and foetus takes place at first through the mesonephros and later through the placenta. Ferm (1956) also comments that the function of the developing definitive placenta resembles adult renal epithelium in that both have the capacity to excrete waste materials and selectively absorb materials of value to the foetus. However, the same author states that the rabbit placenta allows haematrophic nutrition by the 9th day or soon after, at which time the mesonephros is very small and of an early stage of differentiation. Altschule (1930) concluded that the mesonephros, although capable of secreting urine, apparently remains inactive.

Davies (1952), studying the sheep mesonephros, differentiated between proximal and distal segments of the uriniferous tubule on histological grounds and on the distribution of alkaline phosphates. He concluded that the sheep mesonephros functions from the 18th day. The same author (1953) used the P.A.S. method in many species to demonstrate granules in the tubule cells which he thought to be indicative of protein which had crossed the glomerular membrane and had been reabsorbed by proximal tubule cells in both the mesonephros and metanephros.

Gruenwald (1952), using trypan blue experiments, found inception of renal function in the chick mesonephros at 15 days.

Some studies on the possible function of the metanephros before birth may be mentioned. Gruenwald & Popper (1940) found that the most advanced metanephric nephrons are juxtamedullary with a filtration barrier of visceral glomerular capsule epithelium which forms an inextensible sac reducing blood flow and causing coherence of adjacent loops of the glomerular tuft. Rupture of this continuous epithelium in their view occurs at birth and allows function. These workers, in fact, believed that there is a pure mechanical obstruction to metanephric glomerular filtration before birth. Wells (1946) ligatured the ureter *in rats* 6-31 hr. before birth and found that urine is excreted rapidly, but commented on the fact that there are few cases in the literature of stillborns with hydronephrosis or urine in the bladder or both. Smith (1953) observes that human foetal kidneys appear to produce a

urine distinctly dilute and hypotonic as compared with blood plasma, and that this urine is secreted under some pressure.

MATERIAL AND METHODS

Embryos were recovered from does with observed matings of 11, 13, 15, 17, 19 and 23 days previously, and neonate rabbits were also studied. For enzyme preparations fixation was in 80 % alcohol or in absolute alcohol at $-8°$ C. in the deep freeze. Alcoholic Bouin was used for the P.A.S. and Azan methods. The younger embryos were fixed *in toto*; in older specimens the appropriate organ was gently dissected out under a binocular microscope before fixation.

The specimens for enzyme procedures were dehydrated through graduated alcohols in the deep freeze, then cleared in benzene and embedded in paraffin wax using a vacuum embedder. In all cases the specimens were cut at 6μ, and slides to be used later for enzyme localization were kept at $+4°$ C., as were the paraffin blocks before sectioning.

To demonstrate the presence of alkaline phosphatase, either the Martin & Jacoby (1949) modification of the Gomori calcium-cobalt method was used with an incubation time of not more than 20 min., or the azo-dye coupling method similar to that of Pearse (1953) was used with 5·0-chloro-anisidine or Brentamine fast blue B. The latter dye had the advantage of permitting the sections to be mounted in balsam or D.P.X.

Sections were also stained by the azan, Masson trichrome and P.A.S. methods.

FINDINGS

A. 11-day embryos. These varied in size from 6·0 to 7·0 mm. crown-rump length measured before fixation. No metanephros is present at this stage.

The mesonephros is long and relatively narrow as seen in cross-section. The glomeruli are at an early stage of differentiation, the visceral epithelium of the glomerular capsule being 'palisade' in type with a capillary tuft already present, and red-blood cells in that tuft. The tubules are simple, S-shaped and show no morphological differentiation into segments. There is a slight luminal alkaline phosphatase activity of the 'formative' type as described by Rossi, Pescetto & Reale (1953) and Eränkö & Lehto (1954), and slight P.A.S.-positive material in the luminal border. The mesonephric duct also shows slight alkaline phosphatase activity.

B. 13-day embryos. The range of size was 8·5–10 mm. C.R. length. No metanephros is present as yet. The mesonephros is larger than in the 11-day stage and wide in transverse section. The more rostral glomeruli, viz. the oldest (Pl. 1, fig. 2), are fully differentiated with a thin visceral epithelium and large capillaries full of red cells, whilst the caudal glomeruli, viz. the youngest (Pl. 1, fig. 1), still have a thick columnar type of visceral epithelium. The tubules of the more rostral nephrons are fully differentiated into proximal and distal segments with the parietal epithelium of the glomerulus thickening over 4–8 cells at the tubular neck to a columnar type which shows a full alkaline phosphatase (Pl. 1, fig. 4) and P.A.S. activity in their superficial (i.e. luminal) cytoplasm, and this is present throughout the proximal segment. The change from proximal to distal segments also occurs over a few cells,

the epithelium becoming lower, cuboidal in type and with no alkaline phosphatase or P.A.S. activity. The mesonephric duct shows no A.P. activity cranially but is still slightly positive in its caudal part. The associated caudal nephrons are still in the 'formative' stage and show the presence of diffuse but slight alkaline phosphatase activity (Pl. 1, fig. 3).

C. 15-day embryos. These embryos varied in size from 14 to 15·8 mm. C.R. length. The mesonephros is a large body at the height of its activity with *all* the nephrons fully differentiated and none showing any sign of degeneration. The visceral and parietal layers of the epithelium of the glomerular capsule are both thin (Pl. 1, fig. 5), the latter changing at the tubular neck over 4–8 cells to the cuboidal-columnar type of epithelium of the proximal segment. The cells of this segment are large, clear and vacuolated and change sharply to a low cuboidal type in the distal segment. This is shown in Pl. 1, fig. 8. The metanephros is present in the pelvis; it is small, immature and not yet at the nephron-forming stage.

In the mesonephros, the azo-dye and Gomori Ca-Co methods demonstrate a similar distribution of alkaline phosphatase, i.e. in the luminal or brush-border of the proximal segment of the tubule from the tubular neck distally, and this distribution corresponds exactly with that of P.A.S.-positive material. It is equal in all nephrons and is of the maximal 'adult' pattern, i.e. with no signs of degeneration. The distal segments of the tubules are distinguished easily from the proximal segments by their morphology and lack of alkaline phosphatase and P.A.S. activity, the change between segments occurring over 6–10 cells as shown in Pl. 1, figs. 6 and 7. The metanephric tubules show some luminal alkaline phosphatase activity and P.A.S.-positive material, but this is slight, distributed indiscriminately and obviously of a 'formative' and not a 'functional' type.

D. 17-day embryos. The range of size here was from 18·1 to 19·2 mm. C.R. length.

The mesonephros differs but little from that of the 15-day stage, but the cranial (oldest) glomeruli are degenerate. Otherwise it is fully developed and with full alkaline phosphatase activity and P.A.S.-positive reactions in the tubular proximal segments.

In the metanephros which now is larger and situated relatively higher, i.e. more cranially, glomeruli are present in various stages of development, those nearest the medulla being most mature. The visceral epithelium of these is palisade in type and shows slight alkaline phosphatase activity and the tubules are only S-shaped.

E. 19-day embryos. These measured from 25·9 to 28·4 mm. C.R. length.

The rostral one-fifth of the mesonephros is degenerate, the glomeruli showing gross thickening of the basement membrane and they are packed with blood cells; the tubules are disrupted and show loss of continuity, as is evident in Pl. 2, fig. 9. In the degenerate tubules there is still some alkaline phosphatase activity. The remainder of the mesonephros, i.e. the caudal four-fifths, has a normal appearance histochemically and histologically (Pl. 2, fig. 10). The change between the two regions of the mesonephros occurs abruptly, one nephron being normal and the one above it degenerate.

The metanephros now is much larger and, as in the 17-day stage, the majority of the tubules show slight luminal alkaline phosphatase activity (Pl. 2, fig. 9), but a few are strongly positive and show much P.A.S.-positive material. The glomeruli

are larger, some having open capillaries, and the visceral epithelium varies from 'palisade' to the open 'adult' type. (The 'palisade' epithelium is slightly alkaline phosphatase positive.) Some of the nephrons, namely, those near the medulla, have all the 'adult' characteristics regarding structure and histochemistry.

F. 23-day embryos. The range of this litter was 53·5–55 mm. C.R. length.

In all the embryos the mesonephros was present but no longer as a urinary organ.

The metanephros, situated now in the upper lumbar region, shows distinct zones of maturity of nephrons, the youngest being cortical (Pl. 2, fig. 12). The juxta-medullary ones have an adult morphology and histochemistry, viz. a luminal fully positive alkaline phosphatase and P.A.S. reaction in the proximal segment only. The youngest are obviously incapable of function. The P.A.S. reaction shows also masses of positive intracytoplasmic granules in the cells of the collecting ducts as seen in Pl. 2, fig. 11.

G. Neonate. There is a distinct 'zoning' of maturity of glomeruli, the juxta-medullary being 'adult' in type with a thin visceral epithelium and the cortical ones having a thick, palisade visceral epithelium and capillaries containing few or no red cells (Pl. 2, fig. 13). In the older nephrons, the epithelium of the tubular neck thickens up over a few cells and has the characteristic luminal alkaline phosphatase activity and P.A.S.-positive material, as seen in Pl. 2, fig. 14. The proximal segment of the tubule shows these same features histochemically, and the reaction is lost abruptly as the tubule reaches the medulla, i.e. in the proximal loop of Henle. Those proximal tubules associated with glomeruli which are still immature show some slight reaction but to a much lesser degree. P.A.S.-positive material is present as granules in the collecting ducts in addition to the luminal distribution throughout the proximal segment.

The cells of the distal segment are cuboidal in type and show neither P.A.S. nor alkaline phosphatase activity.

DISCUSSION

The capability for function of the mesonephros and metanephros before birth has interested many workers and their criteria of function have varied. Obviously, morphology of the nephron is important and this was emphasized in 1916 by Bremer, who thought that excretion in the rabbit embryo was at first through the mesonephros and later by the placenta. In 1930, Altschule listed some evidence for the function of the human metanephros but thought that although it appeared to be capable of functioning, it apparently did not.

In 1927 and 1928 Hanan and Hurd found that vital dyes were eliminated by the embryonic chick glomeruli and accumulated in the tubules when the brush border was differentiated, i.e. at about 11–12 days. This was an important finding, and in 1937, Gersh published a monograph, again on the excretion of vital dyes. His results, in many species, provided strong evidence for the function of the embryonic and foetal kidneys. By 1940 (Windle) it had been demonstrated that as early as 2½ months in the human, urea is present in the amniotic fluid. In 1946 Wells, working on rats, tied the ureters of the embryos in the last 2 days of gestation and found that the renal pelvis and ureter above the ligature became distended with fluid. He concluded from his results that excretion does not only occur but is rapid and profuse.

The correlation of histochemistry and morphology has been used before in this problem. In 1950, Bradfield had commented that in the brush border alkaline phosphatase is present at a site of active transfer of solutes across cell boundaries, and in all probability this distinctive distribution of the enzyme in the embryonic mesonephros and metanephros is indicative of functional ability. Davies, in a paper in 1952, comments that in the sheep the allantois rapidly increases in size with mesonephric development, and he found evidence from the use of P.A.S. and alkaline phosphatase techniques that the sheep mesonephros is functional from the 18th day. The same author in 1953 described the presence of P.A.S.-positive granules in the tubule cells which he thought to be representative of athrocytosis, i.e. re-absorption of protein droplets. In the adult metanephros, this has been confirmed by Oliver, MacDowell & Yin Chen Lee (1954) and Oliver, Straus, Kretchmer, Yin Chen Lee, Dickerman & Cheriot (1955). By a similar technique Longley & Fisher (1955) have differentiated segments of the nephron in the adult and correlated these differences with differences in function of young and adult animals.

In 1952, Gruenwald described some experiments using trypan blue, and found evidence for inception of renal function in the chick mesonephros at 15 days.

The similarity of distribution of alkaline phosphatase and P.A.S.-positive material has been commented upon by Moog & Wenger in 1952, and this has since been confirmed by many authors. The results here add further confirmation. In 1953 and 1954, papers by Rossi *et al.* and Eränkö & Lehto had drawn attention to the generalized distribution of alkaline phosphatase in embryonic developing tissues—the 'formative' type of distribution—and the localized type—'adult' or 'functional' type—occurring in fully differentiated tissue. That alkaline phosphatase in embryonic tissues could be functional was believed by McKay, Adams, Hertig & Danziger (1955) with reference to many tissues in the human embryo.

Fergusson (1952) by injection of indian ink intravascularly *in vivo* had confirmed the opinion of Gruenwald & Popper (1940) and states that the low glomerular filtration rate in infancy is due to the relatively undeveloped state of the vascular tuft in the outer glomeruli and to the high epithelium of the visceral layer of the glomerular capsule. However, on morphological grounds alone this present investigation has shown that in the rabbit, at any rate, there are numerous fully developed adult nephrons at birth.

It is interesting to compare the results obtained by Wachstein (1955) with our own. He found that tubular cells in human pathological material which have undergone necrobiotic changes still retain varying degrees of alkaline phosphatase activity. This investigation has shown that alkaline phosphatase does linger on in the tubules of degenerating mesonephric nephrons—a marked similarity.

Finally, it must be emphasized that this investigation presents no definite evidence of function of the embryonic and foetal kidneys of the rabbit. It has shown the marked similarities, for example, between 'adult' mesonephric and 'adult' metanephric nephrons. If the one is functional, then it is reasonable to consider that the other at least is capable of function. That the mesonephros should appear coincidentally with the acquisition of a haemotropic placentation, soon after the 9th day, is a mystery. There would seem to be no necessity for a functioning urinary organ other than the placenta, and yet the mesonephros in this animal is large and present

in a fully formed state for some 4–5 days. However, it may be that our views on the placental barrier will have to be modified. Wislocki & Dempsey (1955*a*), in an electron microscope study of the rabbit placenta of 28 days' gestation, suggest that it is haemochrial and not haemo-endothelial in type. The same authors in a paper on the human placenta (1955*b*) state that even in the thinnest places the placental barrier shows no structural similarity to either Bowman's capsule of renal glomeruli or to the alveolar membrane of the lungs. Thus, it may well be that the mesonephros has a vital urinary function to perform.

SUMMARY AND CONCLUSIONS

By histochemical and ordinary staining methods, an attempt has been made to correlate structure and function of the mesonephros and metanephros of the rabbit.

It is concluded that:

1. The mesonephros in the rabbit is a large body present in a fully developed state for approximately 4–5 days (from 13 to 19 days' gestation).

2. In the development of any nephron, mesonephric or metanephric, there is a series of nearly coincident changes:

(*a*) A thinning of the visceral epithelium of Bowman's capsule.

(*b*) A thickening of the parietal epithelium at the tubular neck over 4–8 cells and acquisition of alkaline phosphatase and P.A.S.-positive material from this point throughout the proximal segment of the uriniferous tubule in the luminal border.

(*c*) A distinction morphologically and histochemically between proximal and distal segments.

3. After these changes, no further changes have been observed apart from degenerative ones.

4. It is suggested that these changes are associated with the acquisition of functional capability in the metanephros and therefore must be considered also indicative of a similar capacity in the mesonephros.

5. The metanephros overlaps the mesonephros in the rabbit in capability of function and at birth is not fully differentiated, only the juxta-medullary nephrons being fully formed.

Since this paper was submitted, Davies & Rauth (1947) have reported on the composition of the foetal fluids of the rabbit. They conclude that the origin of the allantoic fluid is wholly or in part a product of the mesonephros, and their findings on the structure of the mesonephric nephron are essentially similar to ours.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. 13-day embryo, caudal end of mesonephros. Glomerulus shows a thick palisade type of visceral epithelium (arrow) but some differentiation of the parietal epithelium at the tubular neck (N). Proximal (P) and distal (D) segments of the tubule can be recognized. Azan. $\times 225$.

Fig. 2. 13-day embryo, cranial end of mesonephros. Glomeruli have a thin, attenuated layer of visceral epithelium (arrows), and heightening of the parietal epithelium at the tubular neck (N) is well marked. Proximal (P) and distal (D) segments of the tubule are fully differentiated. (Note the large arteriole containing many red blood cells entering a glomerulus at A.) Azan. $\times 175$.

Fig. 3. 13-day embryo, caudal end of mesonephros. Glomeruli (G) and tubules (T) with some luminal phosphatase activity. Azo dye method. $\times 190$.

Fig. 4. 13-day embryo, cranial end of mesonephros. Glomeruli (*G*) fully differentiated with a thin layer of visceral epithelium (arrowed). The parietal epithelium thickens up over a few cells at the tubular necks (*N*) to a cuboidal-columnar epithelium and the luminal border shows a strongly positive alkaline phosphatase reaction, continued through the proximal segment (*P*). The distal segment (*D*) has a lower, cuboidal epithelium and shows no alkaline phosphatase activity. Azo-dye, nuclei counterstained with haemalum. ×280.

Fig. 5. 15-day embryo, mesonephros. The glomerulus is fully differentiated with a thin visceral epithelium (arrowed) and a low cuboidal parietal epithelium. The morphological distinction between proximal (*P*) and distal (*D*) segments is well shown. With this staining method, the luminal brush border in the proximal segment is obvious. (See also fig. 2.) Azan. ×380.

Fig. 6. 15-day embryo, mesonephros. The transition between proximal (*P*) and distal (*D*) segments of the tubule occurs over a few cells, the former with a positive alkaline phosphatase reaction. Azo dye. ×375.

Figs. 7 and 8. 15-day embryo, mesonephros. Adjacent sections to show the transition between proximal (*P*) and distal (*D*) segments. Fig. 7, P.A.S. Fig. 8, Masson trichrome. ×430.

Plate 2

Fig. 9. 19-day embryo, mesonephros below, metanephros above. The glomerulus (*G*) and tubule (*T*) in the mesonephros are degenerate. In the metanephros, some tubules show a fully positive alkaline phosphatase reaction. Azo dye. ×110.

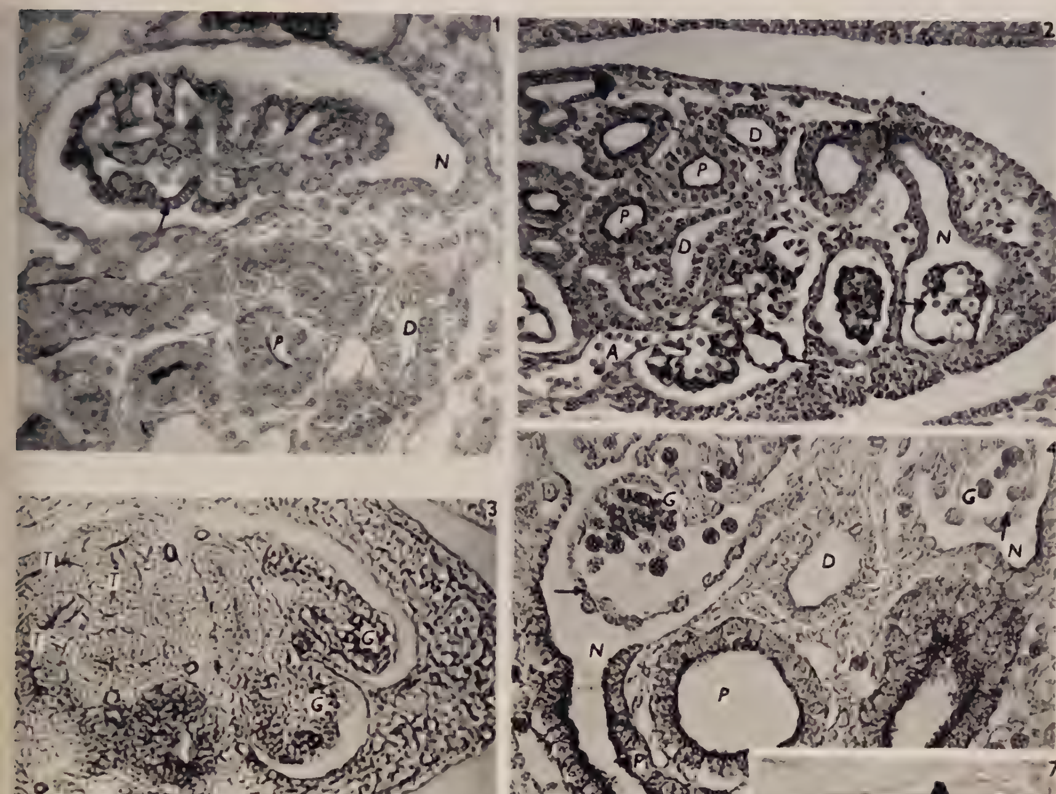
Fig. 10. 19-day embryo, centre of mesonephros. The glomerulus is fully formed and not degenerate, with a thin visceral epithelium (arrowed). The characteristic changes at the tubular neck (*N*) are well shown with proximal segments (*P*) having a positive luminal reaction. P.A.S. ×185.

Fig. 11. 23-day embryo, metanephros. Cortex below, medulla above, the gradation of glomerular development (*G*) being shown, the younger glomeruli being cortical. Proximal segments of the tubules (*P*) show a positive reaction, distal segments (*D*) negative. Note the numerous intracytoplasmic P.A.S.-positive granules (*C*). P.A.S. ×140.

Fig. 12. 23-day embryo, metanephros. Cortex above. The varying maturity of the glomeruli (*G*) is well shown. Azan. ×140.

Fig. 13. Neonate metanephros. Cortex above. The 'zoning' of glomerular maturity is shown (*G*), the oldest (G_3) having an 'adult' morphology. Azan. ×220.

Fig. 14. Neonate metanephros. Cortex above. The distribution of alkaline phosphatase material in the proximal tubular segment is shown, extending from the tubular neck (*N*). Gomori. ×140.



LEESON AND BAXTER—STRUCTURE AND FUNCTION IN MESONEPHROS AND METANEPHROS OF RABBIT.

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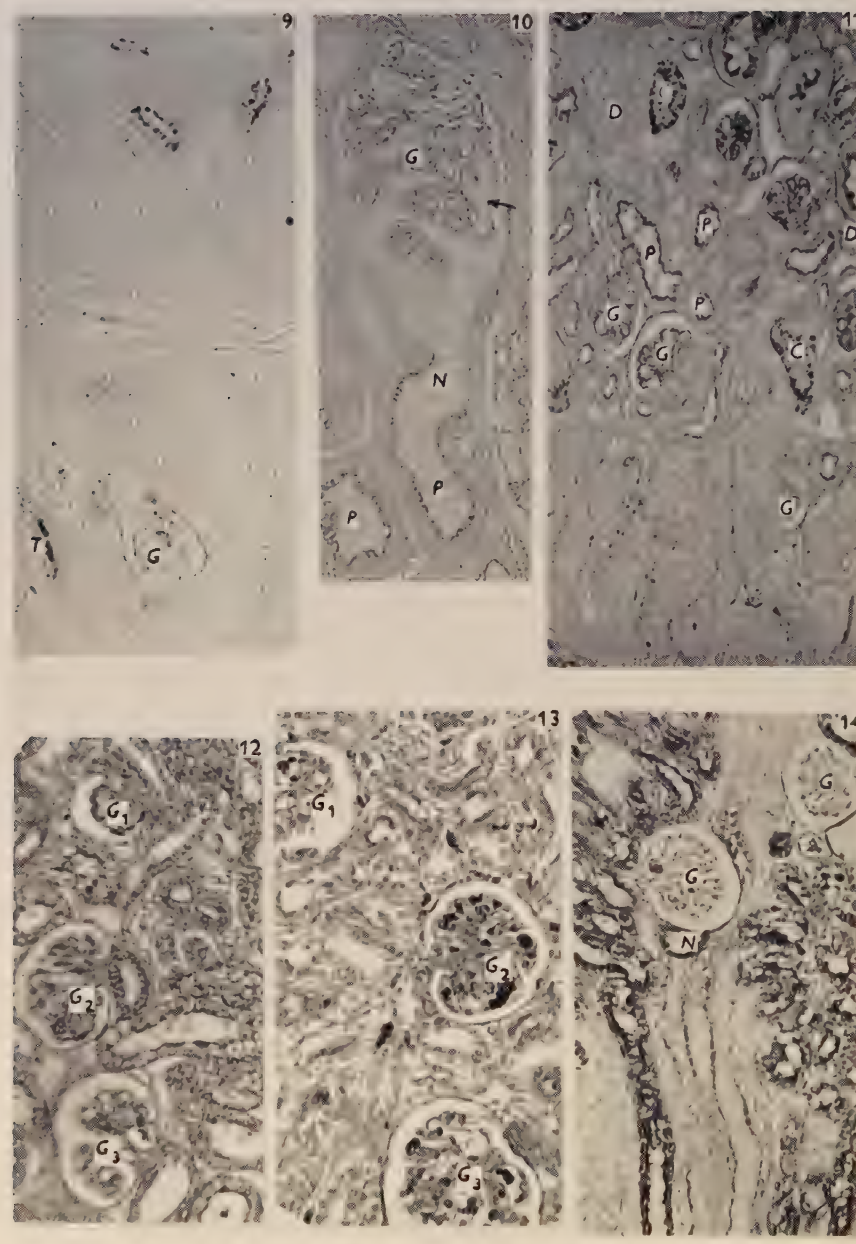

LEESON AND BAXTER—STRUCTURE AND FUNCTION IN MESONEPHROS AND METANEPHROS OF RABBIT

THE DISTRIBUTION OF PROTEIN-BOUND SULPHYDRYL AND DISULPHIDE GROUPS IN VARIOUS TISSUES OF THE VITAMIN A-DEFICIENT RAT

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It is well known that vitamin A deficiency in the rat produces metaplastic changes in the epithelia of various organs and tissues (Wolbach, 1954). This metaplasia is characterized by a regression of epithelia of the columnar or ciliated types to a stratified keratinizing epithelium; epithelia which are normally stratified become hyperkeratinized. Thus it appears that vitamin A is concerned in the maintenance of normal epithelial tissue. The role of vitamin A in this process is not known, but it is possible that a knowledge of the mechanism of the keratinization that occurs in the deficient animal may lead towards an understanding of the biochemical action of the vitamin.

It has been postulated (Giroud & Leblond, 1951; Rothman, 1954) that keratin formation involves oxidation of cysteine sulphydryl groups in adjacent polypeptide chains to produce disulphide linkages. In an attempt to obtain further knowledge of the mechanism of the keratinization process, a study has been made by histochemical means of the distribution of sulphydryl (—SH) and disulphide (—SS—) groups in certain tissues undergoing hyperkeratinization and keratinizing metaplasia in the vitamin A-deficient rat.

MATERIALS AND METHODS

Weanling rats (aged 21 days) of an inbred strain were grouped into pairs of the same litter and sex and were pair-fed on a vitamin A-free diet of the following composition: rice starch, 65 %; casein (Genatosan, low vitamin content), 18 %; Marmite, 8 %; arachis oil, 5 %; salt mixture (Osborne & Mendel, 1913), 4 %. The control animal of each pair received in addition 2250 i.u. vitamin A acetate per week. After 4–5 weeks the animals receiving the diet devoid of vitamin A were showing the usual signs of deficiency, viz. arrested growth and xerophthalmia.

The animals were killed by stunning, followed by bleeding, at various times after the onset of the avitaminotic state. The corresponding control animals were killed on the same day. Tissues were removed immediately and fixed for 24 hr. in a 1 % (w/v) solution of trichloroacetic acid in 80 % ethanol. The tissues were then dehydrated, embedded in paraffin and sectioned at 8 μ. For the demonstration of protein-bound —SH and —SS— groups, the histochemical procedures described by Barrnett & Seligman (1952, 1954) were followed. The method depends on the reaction of the —SH group with 2:2′-dihydroxy-6:6′-dinaphthyl disulphide to form a colourless substance which is then converted into an intensely coloured azo dye by coupling

with tetrazotized *o*-dianisidine. A red colour (monocoupling) is taken to indicate a sparse, widely separated distribution of —SH groups, whereas a blue colour (dicoupling) indicates a higher concentration of —SH groups. The distribution of —SS— groups was determined by blocking the —SH groups with iodoacetic acid and then splitting the —SS— linkages with potassium cyanide to form —SH groups. The section was then treated with the staining reagents. Since the blocking of the —SH groups was not always complete (cf. Goldblum, Piper & Campbell, 1954), it was necessary to prepare a so-called —SH-negative section which was stained after it had been treated with the blocking agent. This section was then compared with the cyanide-treated —SS— section in order to determine the true distribution of —SS— groups.

Sections were also stained by the standard haemotoxylin-eosin method.

RESULTS

General observations

The external signs of vitamin A deficiency such as loss of body weight and xerophthalmia appeared in all animals which had received the vitamin A-free diet. The animals usually reached a weight plateau after approximately 30 days on the diet; 5–6 days later xerophthalmia appeared and the animals had then begun to show a weight decline.

An interesting feature of the deficient animals was the frequent occurrence of grossly distended stomachs. These were filled with fluid and undigested food material and the blockage appeared to be in the region of the pyloric sphincter. The occurrence of distended stomachs in vitamin A-deficient rats has been noted previously by Mayer & Krehl (1948) and Heaton, Lowe & Morton (1955).

Effect of deficiency on epithelial tissues and organs

Tissues and organs of eleven vitamin A-deficient rats were examined and compared with those of the pair-fed controls. The tissues and organs which were selected for detailed examination were as follows: oesophagus, forestomach, cornea, vagina, skin, bladder, trachea and salivary glands. It was found that in all the regions normally lined by stratified squamous epithelium, except skin, a hyperkeratinization occurred in the deficient animals. In the case of the bladder, trachea and salivary glands, metaplastic changes were found only in animals which had been kept for a longer period in the deficient state (11–14 days from the onset of xerophthalmia). Thus it appears that hyperkeratinization preceded the metaplastic changes.

The epithelial changes found corresponded in general to those described by other workers (reviewed by Wolbach, 1954), and can be summarized briefly as follows: In the oesophagus and forestomach there was a thinning of the basal layers of the epithelium and an increase in number and a loosening of the layers of the stratum corneum. Results were similar to those obtained by Planel, Sardou & Guilhem (1955). In the cornea the number of layers in the stratified squamous epithelium was increased with an accompanying keratinization of the superficial layer to produce a well-marked stratum corneum. In the vagina there was a non-cyclical cornification of the epithelium. The skin of the ventral abdominal wall was not markedly

affected, but the total thickness was decreased owing to a loss of fat (Ramalingaswami & Sinçlair, 1953). It is noted that Loewenthal (1956) also did not find hyperkeratinization of the skin of the vitamin A-deficient mouse. In the bladder there was keratinization of the transitional epithelium and in the trachea nests of flattened cells were found among the columnar cells. The ducts of the salivary glands showed a dilatation with a keratinization of the epithelium; inflammatory cells were seen in the lumen.

Distribution of sulphydryl and disulphide groups

Cornea. The normal cornea is lined by a non-keratinized stratified squamous epithelium and the —SH groups were found evenly distributed throughout the layers of the epithelium (Pl. 1, fig. 1). —SS— groups were not present, since the reaction for —SS— was the same as that for an —SH-blocked section treated for —SH alone (Pl. 1, figs. 2, 3).

In the vitamin A-deficient animals in which marked cornification was found, —SH groups were found throughout the epithelium, with a high concentration in the intra-epithelial zone, which appears to correspond to the stratum lucidum and the lower layers of the stratum corneum (Pl. 1, fig. 4). —SS— groups were absent from the Malpighian layers but were found in fair quantity in the stratum corneum (Pl. 1, fig. 5). They were not usually particularly concentrated in the intra-epithelial zone.

Bladder. The transitional epithelium of the normal bladder showed a distribution of —SH groups (Pl. 1, fig. 6) similar to that of the normal corneal epithelium; i.e. they occurred evenly distributed throughout the layers. As in the cornea, no —SS— groups were demonstrated (Pl. 1, fig. 7).

In the bladder of one deficient animal different degrees of metaplasia were found. In parts, the epithelium was transitional in character but with an increase in the number of layers. Some of the outermost cells in this region were flattened and showed an acidophil cytoplasm. No —SS— groups were found but —SH groups occurred throughout the epithelium. In other parts there was a highly cornified epithelium with a very thick stratum corneum. —SH groups were present throughout the epithelium with a high concentration in the intra-epithelial zone (Pl. 1, fig. 8). —SS— groups were found throughout the stratum corneum and the intra-epithelial zone; in some regions of the latter, an apparent high concentration of —SS— groups was observed, but this was due to incomplete blocking of the —SH groups (Pl. 1, fig. 9).

Oesophagus and forestomach. Both the normal and vitamin A-deficient animals showed a similar pattern of distribution of —SH and —SS— groups in these areas. —SH groups were found throughout the epithelium with a high concentration in the intra-epithelial zone, whereas —SS— groups were absent from the Malpighian layers but present in the rest of the epithelium (Pl. 2, figs. 10–17). In the forestomach, and occasionally in the oesophagus, the —SH in the intra-epithelial zone was resistant to the blocking agent and often a well-marked reaction was found both in the —SH-blocked and —SS— sections (Pl. 2, fig. 17).

Vagina. The distribution of —SH and —SS— groups in the vaginal epithelium of the vitamin-A deficient rat was similar to that found in other keratinized epithelia

(Pl. 2, figs. 18, 19). The pattern of —SH-group distribution was similar to that described by Kahn (1954) for the vaginal epithelium of the castrate rat treated with oestrogen and by Asscher & Turner (1955) for the oestrous vagina of the mouse; the latter authors also found an intense —SH-group reaction in the region of the stratum granulosum. It should be noted that in vaginal sections treated for —SS—, the blockage of —SH groups was always complete.

Thus, with normal animals, it is seen that in the regions examined the distribution of —SH groups in stratified squamous epithelia was found to be similar to that described by Barrnett (1953). —SH groups were distributed throughout the stratum granulosum and the stratum corneum. The presence of a marked band of intense reaction in the deeper layers of the stratum corneum in certain tissues (oesophagus and skin) was also confirmed. —SS— groups were absent from the nucleated layers of the epithelia but they were present in the stratum corneum.

In the vitamin A-deficient animals in the regions which are normally lined by a stratified squamous epithelium, the distribution of the —SH and —SS— groups was similar to that in the normal animals, but the width of the band of high —SH-group reactivity in the intra-epithelial zone was often reduced. In the areas undergoing keratinizing metaplasia the distribution of both groups conformed to the pattern found in the hyperkeratinized epithelia.

DISCUSSION

The widely accepted concept that keratinization in epithelial tissues involves a complete oxidation of —SH to —SS— above the Malpighian layer (Giroud & Leblond, 1951) is not substantiated by the results of the present investigation. In addition to —SS— groups, —SH groups were found in appreciable concentrations in the cornified layers of stratified squamous epithelia of normal animals and of epithelia undergoing hyperkeratinization and keratinizing metaplasia in vitamin A-deficient animals. Thus these results have confirmed and extended the work of Barrnett (1953) and Eisen, Montagna & Chase (1953), who found that —SH groups were present in the stratum corneum of normal epithelial tissue. However, it was confirmed that in the formation of hair keratin there is a complete oxidation of —SH to —SS—, since there was a complete absence of —SH in the fully formed hair cortex but an intense reaction for —SS— (cf. Barrnett, 1953; Eisen *et al.* 1953).

The occurrence of a deeply staining —SH layer in the region of the stratum lucidum and the deeper layers of the stratum corneum is interesting, and it is possible that it may be analogous to the keratogenous zone of the hair shaft as suggested by Eisen *et al.* (1953). Is this region of high concentration of —SH groups due to a close packing of the prekeratinized cells or to an active accumulation of —SH containing protein? In studies on keratin formation in normal tissues using ^{35}S-labelled cystine, Bern, Harkness & Blair (1955) found that radioactivity was greatest in the region immediately below the keratin layer in the keratinized epithelium of the vagina, tongue, foot-pad, oesophagus and forestomach; this region of high ^{35}S concentration appears to correspond to the region of high —SH concentration as revealed histochemically. These authors considered the possibility that this higher radioactivity could be due to a flattening of the cells and decrease in their volume prior to keratinization. However, the activity is present within a short time after

the administration of radioactive cystine suggesting that it need not be ascribed to the ^{35}S content of the cells which have proliferated from the basal layer. It was concluded therefore that keratinization is an active synthetic process as indicated by Biggers (1953).

The present results have thus added to a growing amount of evidence which makes it necessary to review the concepts of keratinization in epithelial tissues. Van Scott & Flesch (1954) have advanced a new hypothesis based on chemical analyses of —SH and —SS— concentrations in human skin. They found practically identical concentrations of —SS— in the Malpighian layer and the stratum corneum with little evidence for a conversion of —SH into —SS—, and suggested that an —SS— containing substance already formed in the Malpighian layer gives rise directly to the keratinous substance of the horny layer. However, in the present investigation, the presence of —SS— groups in the Malpighian layer was not confirmed; they were found only in the stratum corneum. It seems likely, therefore, that although there is a conversion of —SH into —SS— groups above the Malpighian layer, the conversion is an incomplete one, and substantial quantities of —SH groups remain in the keratin layer.

Because of the difficulty of interpreting the results quantitatively it was not possible to determine whether the relative concentrations of —SH and —SS— groups were affected in the hyperkeratinized epithelia of the vitamin A-deficient animals. The pattern of the distribution of the two groups in these epithelia was similar to that of the normal keratinized epithelia. It was found, however, that the width of the band of high —SH-group concentration in the intra-epithelial zone was reduced in the hyperkeratinized tissues. A similar result was noted by Bern, Elias, Pickett, Powers & Harkness (1955), who found that the —SH-group reaction in this zone was intensified in rat skin after the external application of vitamin A. It is possible that this effect could be due to the accelerated rate of conversion of —SH into —SS— groups which probably occurs in hyperkeratinization. On the other hand, it could also be explained as an apparent difference owing to the loosening of the layers of the stratum corneum in the more highly keratinized epithelia.

SUMMARY

1. A comparison has been made of the histochemical distribution of protein-bound sulphydryl (—SH) and disulphide (—SS—) groups in the epithelia of various regions in young normal and vitamin A-deficient rats.

2. —SH groups, but not —SS— groups, were found in normal non-keratinized epithelia. In keratinized epithelia in normal animals and epithelia undergoing hyperkeratinization and keratinizing metaplasia in vitamin A-deficient animals, —SH groups were distributed throughout all the layers, but —SS— groups were present only in the keratin layers.

3. A constant finding in keratinized epithelia was a high concentration of —SH groups in the region corresponding to the stratum lucidum and the lower layers of the stratum corneum.

4. The results do not substantiate the concept that epithelial keratinization involves a complete oxidation of —SH to —SS— groups immediately above the Malpighian layer.

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EXPLANATION OF PLATES

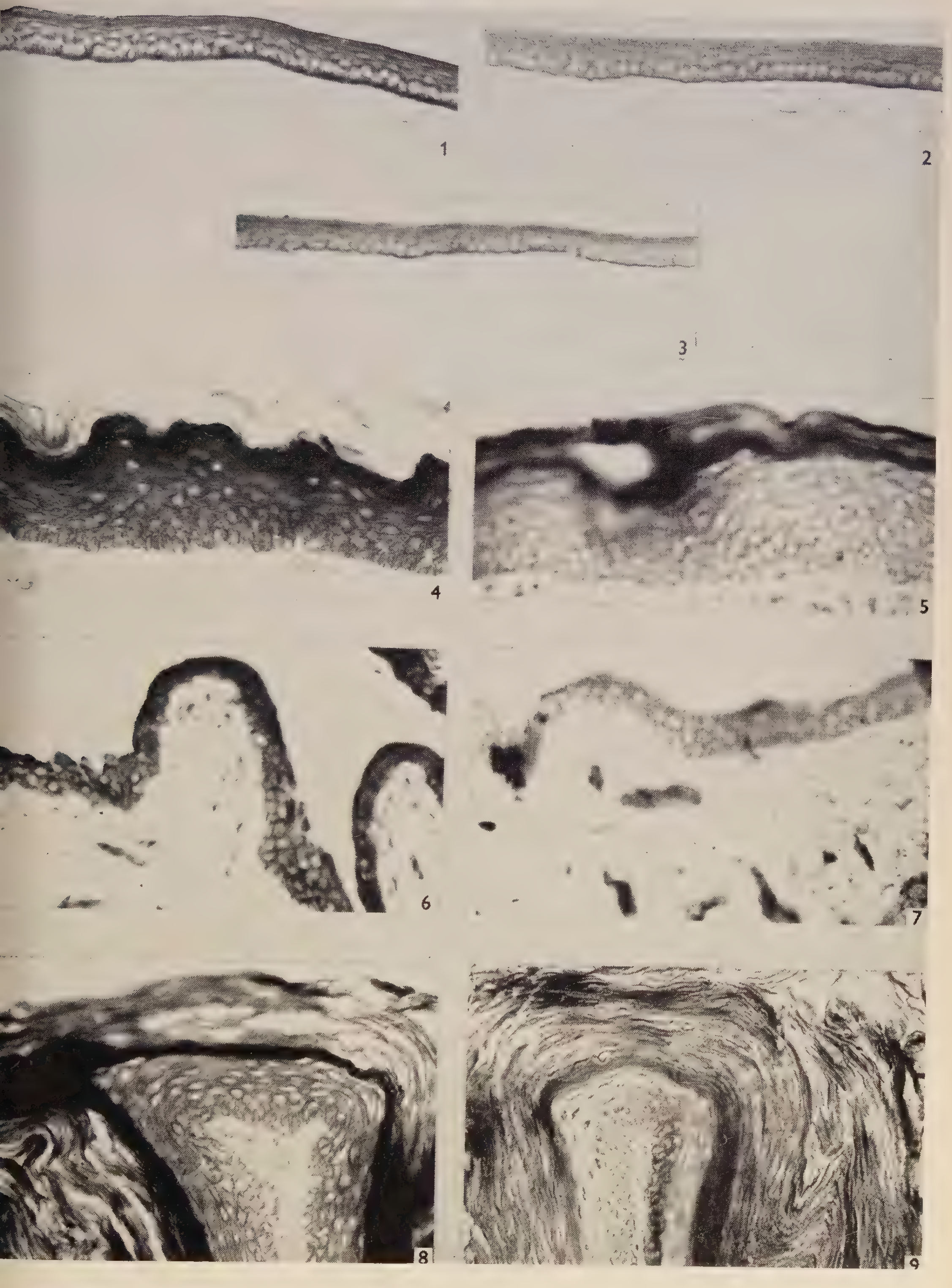
All sections were stained by the Barrnett & Seligman method for protein-bound sulphydryl or disulphide groups. ×180. Ilford green filter (4850–6000Å.).

Plate 1

Fig. 1. Corneal epithelium of normal rat stained for —SH groups. Note that they are distributed throughout the epithelium.

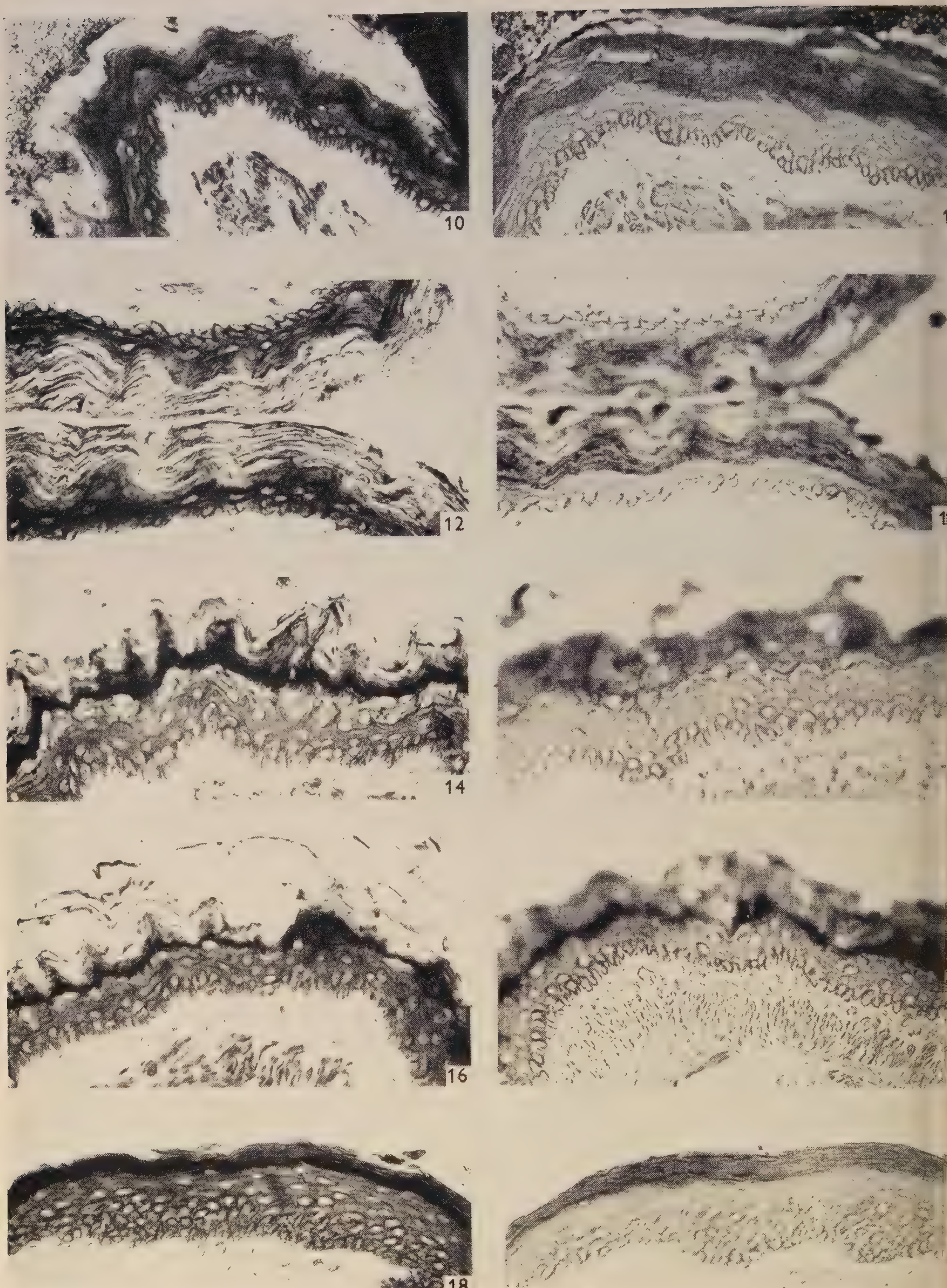
Fig. 2. Corneal epithelium of the same rat treated for —SS— groups. Compare with fig. 3.

Fig. 3. Corneal epithelium of the same rat stained for —SH after blocking of the —SH groups. Note that the blocking of the —SH groups is incomplete and that the intensity of stain is the same as in the slide treated for —SS— groups (fig. 2).



REDFEARN AND STRANGEWAYS—DISTRIBUTION OF PROTEIN-BOUND SULPHYDRYL AND DISULPHIDE GROUPS

(*Facing p.* 396)



REDFEARN AND STRANGEWAYS DISTRIBUTION OF PROTEIN-BOUND SULPHYDRYL AND DISULPHIDE GROUPS

Fig. 4. Corneal epithelium of a vitamin A-deficient rat stained for —SH. Note the distribution of the —SH groups throughout the keratinized epithelium.

Fig. 5. Corneal epithelium of the same vitamin A-deficient rat treated for —SS— groups; they are found in the stratum corneum but are absent from the Malpighian layers.

Fig. 6. Bladder epithelium of normal rat showing —SH groups distributed throughout the epithelium.

Fig. 7. Bladder epithelium of the same rat treated for —SS— groups. No —SS— groups are present as the same intensity of staining was shown in the —SH-blocked slide stained for —SH.

Fig. 8. Bladder epithelium of a vitamin A-deficient rat. —SH groups stained. Note the metaplasia of the epithelium and the occurrence of the —SH groups throughout the layers.

Fig. 9. Bladder epithelium of the same vitamin A-deficient rat treated for —SS— groups. They are confined to the stratum corneum. Note regions of an apparent high concentration of —SS— groups owing to incomplete blocking of —SH groups.

PLATE 2

Fig. 10. Epithelium of oesophagus of normal rats showing —SH groups throughout the epithelium with a concentration in the intra-epithelial zone.

Fig. 11. Epithelium of oesophagus of the same rat treated for —SS— groups. They are found in the stratum corneum only, as the apparent staining in the Malphighian layers was found in the —SH-blocked slide.

Fig. 12. Epithelium of oesophagus of a vitamin A-deficient rat stained for —SH. The —SH groups are present throughout the epithelium. Note the decrease in the number of layers in the Malpighian layer.

Fig. 13. Epithelium of the same vitamin A-deficient rat showing the presence of —SS— groups in the stratum corneum only.

Fig. 14. Epithelium of the forestomach of a normal rat stained for —SH groups. They are distributed throughout the layers with a concentration in the intra-epithelial zone.

Fig. 15. Epithelium of the forestomach of the same rat showing —SS— groups confined to the stratum corneum.

Fig. 16. Epithelium of the forestomach of a vitamin A-deficient rat stained for —SH. —SH groups are present throughout the layers with a concentration in the intra-epithelial zone. Note the thinning of the Malpighian layer.

Fig. 17. Epithelium of the forestomach of the same vitamin A-deficient rat treated for —SS— groups. The groups are present in the stratum corneum and the apparent concentration in the intra-epithelial zone is due to incomplete blocking of the —SH groups before staining for —SS—.

Fig. 18. Vaginal epithelium of a vitamin A-deficient rat stained for —SH. The —SH groups are distributed throughout the epithelium.

Fig. 19. Vaginal epithelium of the same vitamin A-deficient rat treated for —SS—. Note that the —SS— groups are confined to the stratum corneum.

SULPHATED MUCOPOLYSACCHARIDES IN RODENT TEETH

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The localization of mucopolysaccharides in teeth has been the subject of several recent investigations. Using chemical methods Stack (1951, 1954) showed that dentine contained 0·2% by weight of mucopolysaccharide, and that enamel contained a glycoprotein. Hess & Lee (1952) isolated chondroitin sulphuric acid, as its potassium salt, from dentine with a yield of 0·64%. The same authors demonstrated the presence of galactosamine in dentine by paper chromatography.

Histochemical methods were used by Wislocki & Sognnaes (1950). Acid mucopolysaccharides were detected by metachromatic staining reactions by Sognnaes (1955), and Bevelander & Johnson (1955) distinguished between acid mucopolysaccharides and neutral mucopolysaccharides, again by histochemistry.

The technique of autoradiography was employed by Bélanger (1954, 1955) to show that, in the experimental animal, injected S35 as sulphate was incorporated in dentine and enamel. Leblond, Bélanger & Greulich (1955) have studied the problem of calcification in bones and teeth, and used autoradiography to localize injected isotopes including S35.

The present work follows on the description given by Curran & Kennedy (1955*a*) of a close correspondence between the localized uptake of inorganic S35 *in vivo*, and the presence, at the sites of uptake, of mucopolysaccharides as revealed by the usual staining methods. It was suggested that the autoradiographic method is an effective means of identifying specifically the presence in tissues of sulphated mucopolysaccharides.

MATERIALS AND METHODS

S35 as sulphate at pH 7 was administered intraperitoneally or subcutaneously to a total of thirty-seven small rodents (Table 1). In the case of the foetal mice the mothers were given the isotope subcutaneously. The dose of S35 ranged from 1 to 5 μc./g. body weight except for the foetal mice where the fraction of the maternal dose which crossed the placenta was not determined.

The animals were killed with chloroform 5–169 hr. after injection. The foetal mice were fixed intact in a mixture of equal parts of alcohol and acetone for 2 hr. before slicing in the coronal plane. Fixation of the slices was continued for a further 6 hr. Thin blocks were taken from the jaws of the older animals, usually in the sagittal plane, and fixed for 24 hr. in a mixture of equal parts of alchohol and acetone. Alcoholic formalin, absolute alcohol, and 10% neutral formalin were equally satisfactory. Five per cent formic acid for up to 72 hr. was used for decalcification. Embedding in 2% celloidin for 24 hr. was used prior to 1½ hr. vacuum embedding in

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paraffin. Sections 5–6μ thick were floated on water at 40° C. and those required for autoradiography were mounted on gelatine-coated slides.

The autoradiographs were prepared and stained with light green and safranin O as described previously (Curran & Kennedy, 1955*a*). Two grades of stripping emulsion were used: the Kodak standard autoradiographic plate, and a coarser grain more sensitive Kodak experimental plate, type V1001.

Sections adjacent to those used for autoradiography were stained with alcian blue (Pearse, 1953), by the alcian blue-chlorantine fast red method of Lison (1954), by the periodic acid-Schiff technique, and for metachromasia with toluidine blue at pH 2·6 and 4·9. It was not assumed that any single method was strictly specific for mucopolysaccharides.

Table 1. *Animals used and range of time interval between injection of S35 and sacrifice*

Animals	No.	Time interval (hr.)
Adult albino mice	11	15–69
Suckling albino mice	9	5–169
Foetal albino mice	3	5–19
Adult white rats	1	19
Suckling white rats	12	5–169
Adult guinea-pigs	1	24

Table 2. *Uptake of S35 in adult animals estimated by intensity of autoradiographic images*

Tissue	Interval between injection of S35 and sacrifice: 15–19 hr.	20–24 hr.	64–69 hr.
Dental pulp	±	+	+
Odontoblasts	±	0	±
Predentine	++	++	++
Dentinal matrix	0	0	0
Enamel matrix	0	+	±
Ameloblasts	±	±	±

The intensity of the images is graded as follows: 0, no uptake; ±, occasional weakest uptake; +, weakest uptake; ++, intermediate uptake; +++, greatest uptake.

RESULTS

Autoradiography

(1) *Adult animals.* The distribution of S35 and the intensity of the autoradiographic images varied with the age of the animal and with the time interval between injection of the isotope and the death of the animal. In the adult animal the time factor was associated with the least variation in the intensity and in the uptake pattern; the distribution was relatively constant (Table 2).

The highest uptake was found uniformly in the predentine and no species difference was noted (Pl. 1, fig. 1). The maximum uptake was less than the maximum found in young animals (Table 3). The cells and matrix of the dental pulp showed a rather diffuse uptake in most preparations, but were negative in the oldest teeth in which the pulp cavity was smallest. There was occasional focal activity over cells of the

dental pulp. No activity was seen in the dentinal matrix, and the odontoblasts, enamel matrix, and ameloblasts showed only an occasional weak uptake.

(2) *Foetal and suckling animals.* The uptake pattern was more complex in this group than in the adult. The maximum intensity was found consistently in predentine, in dentinal matrix, and in the dental pulp. The variation in the uptake pattern with time was studied in the following structures (Table 3).

Dentinal matrix. The spatial distribution of radioactivity in this tissue varied most with the time interval between injection and sacrifice. Between 5 and 19 hr. after injection the autoradiographs showed a dense band of activity in the predentine (Pl. 1, fig. 2). As the time interval increased, the intensity of this image over the predentine decreased, and a further band of activity appeared in the dentinal matrix. This active zone in the dentinal matrix increased in intensity with time up to 135–139 hr. after injection. The intensity decreased in the 164–169 hr. period.

Table 3. *Uptake of S 35 in foetal and suckling animals estimated by intensity of autoradiographic images*

	Interval between injection of S 35 and sacrifice							
Tissue	5–9 hr.	10–14 hr.	15–19 hr.	64–69 hr.	90–94 hr.	115–119 hr.	135–139 hr.	164–169 hr.
External enamel epithelium	++	+	++					
Internal enamel epithelium	++	+	++					
Stellate reticulum	+ to ++	+	+ to ++					
Dental papilla	++	+	+					
Dental pulp	++	++	++	++	++ to +++	++ to +++	++	++ to +++
Odontoblasts	++	± to ++	± to ++	+	± to +	0	0	0
Predentine	+++	+++	+++	++	++	+	+	+
Dentinal matrix	+	+	+	+	++	+++	+++	++
Enamel matrix	++	++	++	+	++	++	++	+
Ameloblasts	++	± to ++	± to ++	± to +	+	0	0	0
Stratum intermedium	++	+	+	± to +	+	±	0	0

Where blank spaces occur in the table no examination of the appropriate tissue was made at the time tabulated. The symbols 0, ±, +, ++ and +++ have the same significance as in Table 2.

The band also changed its position in the matrix. As the time interval increased the zone of activity was found progressively farther out towards the amelo-dentinal junction, and 164–169 hr. after injection its edge coincided with the amelo-dentinal junction (Pl. 1, figs. 3, 4). The movement outwards, however, was not uniform in all parts of the dentinal matrix, but was most rapid towards the apex. For example, 90–94 hr. after injection, at the crown of a molar, the active band was found to be about equidistant from the amelo-dentinal junction and the predentine, while, at the apex, it had reached the amelo-dentinal junction. In many cases, in sections of incisors, the uptake in the dentinal matrix appeared on the convex sides of the teeth as two closely apposed bands, and on the concave sides as one band (Pl. 1, fig. 5). The displacement outwards with time was again as described above. An appreciable uptake was still apparent, up to 169 hr. after injection, in the predentine, with frequently a clear band of little or no activity between the predentine and the densely active zone in the dentinal matrix. The activity in the dentinal matrix generally could not be related to the dentinal tubules, but appeared as quite sharply

demarcated zones fairly constantly about the breadth of the layer of odontoblasts.

Enamel matrix. No such marked zoning of activity was seen. When a positive uptake occurred it appeared as a uniform stippling over the whole thickness of the matrix (Pl. 2, fig. 6). This band was sometimes noted to be darkest at the apex of an incisor, and to shade off in intensity towards the incisal tip. Some apparent increase in activity was also noted, at times, in the enamel matrix near the layer of ameloblasts. This finding, however, was not the general rule and was due to a darker staining reaction in the section rather than to an increase in the intensity of the autoradiographic image in the overlying emulsion. No definite variation in intensity in the enamel matrix uptake was observed with time. At 164–169 hr. there was a slight decrease, as in the case of the dentinal matrix uptake, but it cannot be claimed in either case that this marked a turning point.

Odontoblasts and ameloblasts. The cells showed activity up to 94 hr. after injection (Pl. 2, figs. 7, 8). The activity was rather diffuse and, at times, weak, but in the ameloblasts at 10–14 hr. it was possible in some cases to localize the maximum activity in the poles of the cells adjacent to the enamel matrix (Pl. 2, fig. 9). When such a localization was possible it was noted that it occurred in large, elongated ameloblasts. The stratum intermedium and the membrane between it and the ameloblasts both showed a fairly constant uptake for the period 5–94 hr. after injection (Pl. 2, figs. 7–9). As for the odontoblasts and ameloblasts this activity was not detected in the period 115–169 hr. after injection.

Dental pulp. Radioactivity was present throughout the whole period of observation, and the intensity of the uptake showed a tendency to increase with time. Occasionally focal activity over cells of the dental pulp was noted (Pl. 2, fig. 7), but generally the uptake was diffuse over cells and matrix. The maximum uptake was often seen at the apical part of the pulp, and this sometimes corresponded with an increased staining reaction for mucopolysaccharide. This localization was not constant and, particularly for the period 115–119 hr. after injection, the maximum activity was found in the central part of the pulp, and shaded off towards the odontoblasts in which no activity was seen at this time.

Other structures. In the earliest stages of the developing tooth the internal and external enamel epithelia, the stellate reticulum, and the dental papilla showed a fairly constant uptake, up to 19 hr. after injection. Activity in the dental papilla was usually greatest in the regions of future cusp development. In many cases focal cellular activity was noted in the stellate reticulum as well as more diffuse stippling over cells and matrix (Pl. 2, fig. 10). At the bud stage the surviving connexion to the dental lamina showed weak activity with a heavier uptake in the mesodermal tissue on either side of the stalk. The squamous epithelium was inactive. At a later stage, the dental follicle showed S35 uptake which was also noted in the early periodontal membrane. The activity in these foetal and neo-natal structures was not observed, because of technical difficulties, for periods greater than 19 hr. after injection.

No species variation in uptake was noted in this group of animals.

Staining reactions

The staining reactions of the dental tissues showed quite marked variation by the methods used, and generally did not give as consistent a picture as the uptake pattern of S35 in corresponding sections (Table 4).

Metachromasia was fairly consistently demonstrated in the matrices of the stellate reticulum, the dental papilla and the dental pulp. Predentine, dentinal matrix and enamel matrix, however, were more variable. The peripheral parts of the dentinal tubules only stained metachromatically when the toluidine blue was buffered to pH 4·9 and were orthochromatic at pH 2·6. Weakly staining intracellular metachromatic material was seen occasionally in the supranuclear part of the ameloblasts. A similar weak reaction was demonstrable in a few sections in the odontoblasts. The cells of the dental papilla, dental pulp and stellate reticulum were

Table 4. *Staining reaction of dental tissues compared with S35 uptake in foetal, suckling and adult animals*

	Staining method					
Tissue	1	2	3	4	5	S35 uptake
External enamel epithelium	Variable	+ve	+ve	+ve	Variable	+ve
Internal enamel epithelium	Variable	+ve	+ve	+ve	Variable	+ve
Stellate reticulum (cells and matrix)	+ve	+ve	Variable	Variable	+ve	+ve
Dental papilla (cells and matrix)	+ve	+ve	+ve	+ve	+ve	+ve
Dental pulp (cells and matrix)	+ve	+ve	+ve	Variable	+ve	+ve
Odontoblasts	Variable	+ve	Variable	Variable	Variable	+ve
Dentinal tubules (peripheral part)	+ve	+ve	Variable	−ve	+ve	Not determined
Predentine	Variable	−ve	Variable	−ve	+ve	+ve
Dentinal matrix	−ve	−ve	Variable	Variable	−ve	+ve
Enamel matrix	Variable	Variable	Variable	Variable	−ve	+ve
Ameloblasts	−ve	+ve	Variable	Variable	Variable	+ve
Stratum intermedium	Variable	+ve	+ve	−ve	−ve	+ve

Staining methods are numbered as follows: 1, alcian blue; 2, alcian blue-chlorantine fast red; 3, periodic acid-Schiff; 4, toluidine blue at pH 2·6; 5, toluidine blue at pH 4·9. No quantitative significance is attached to the symbols in this table. +ve, positive; −ve, negative.

more frequently metachromatic, particularly at the higher pH used. Variable results were again obtained in the external and internal enamel epithelia, and the stratum intermedium was consistently orthochromatic.

Mucopolysaccharide methods were quite uniformly positive for the cells and matrix of the dental papilla and dental pulp, and in the large majority of cases for the cells and matrix of the stellate reticulum. The predentine, dentinal matrix and enamel matrix gave extremely variable results, particularly with the periodic acid-Schiff technique. The dentinal tubules, however, consistently stained for mucopolysaccharide by the alcian blue method. The positive result here was in the peripheral parts of the tubules, and frequently only for that portion of their length occupying the predentinal layer. The odontoblasts, ameloblasts, stratum intermedium, and internal and external epithelia did not give consistent results, particularly with alcian blue.

In the staining reactions generally no species difference was observed, and the age of the animal did not cause as much variation in the results as it did in the case of the S35 uptake studies. Only in the case of the dental pulp was it possible to grade the staining reaction and it corresponded closely to the S35 uptake grading. This was most evident with alcian blue and toluidine blue. Comparison of staining reactions of decalcified and undecalcified material showed no significant differences, although a tendency was noted for metachromasia to appear more frequently in the enamel and dentinal matrices of decalcified teeth. This, however, was not a constant finding.

DISCUSSION

Chemistry. Reference has already been made to the isolation of chondroitin sulphate from dentine by Hess & Lee (1952). They also showed that the chondroitin sulphate from dentine contained galactosamine, thus indicating its similarity to the chondroitin sulphate of cartilage which is a complex of acetylgalactosamine, glucuronic acid and sulphuric acid (Bray, Gregory & Stacey, 1944). Earlier work by Pincus (1949) led to the description of polysaccharide components of dentine and enamel, which, when exposed to sulphatase, may release sulphuric acid. Pincus stated that the polysaccharide component of dentine is chondroitin sulphate, and that of enamel may be mucoitin sulphate.

Our autoradiographic results were obtained mainly in decalcified preparations, and, therefore, it is very probable that the S35 localized was present in organic form. Any soluble inorganic sulphate present, even in undecalcified tissues, is lost in histological processing and washing prior to autoradiography. Tarver & Schmidt (1939) showed that there was no significant incorporation of inorganic S35 in cystine, and in methionine Boström and Åqvist (1952) showed that again inorganic S35 was not significantly utilized. Taurine showed a very low uptake. Although Sognnaes (1955) suggested that there may be at least four different sources of organically bound sulphur in enamel (SH, SS, CH_3S, SO_4), and three in dentine (SH, CH_3S, SO_4), it is not probable, on the evidence available, that inorganic sulphate is bound by the sulphur containing amino-acids *in vivo*. Curran & Kennedy (1955*a*) showed, by autoradiographic studies, that inorganic S35 was not taken up by tissues containing sulphur compounds other than ester sulphates. There was some evidence that a trace of radioactive cystine was synthesized by intestinal bacteria, but the amount was negligible in the autoradiographs. It is thus probable, when a positive autoradiographic uptake of S35 is demonstrated after the usual histological processing of the tissue, that the S35 is incorporated in a sulphomucopolysaccharide or in a sulphated precursor of a sulphomucopolysaccharide. Inorganic radioactive sulphate labels sulphated mucopolysaccharides or their precursors *in vivo*. The present work supports this hypothesis.

Histochemistry. The extracellular dental tissues in which bound S35 has been demonstrated by autoradiography have been shown to stain more or less uniformly for acid mucopolysaccharides (Bevelander & Johnson, 1955). Wislocki & Sognnaes (1950) reported variability in the periodic acid-Schiff reaction in enamel and stellate reticulum. Sognnaes (1955) summed up histochemical observations as favouring the interpretation that a Schiff-positive acid mucopolysaccharide component is

present in the ground substance of the stellate reticulum, of the dental papilla, and of the dental sac, in the interprismatic regions of the enamel, and in the peripheral regions of the dentinal tubules. Our observations confirm the correspondence between histochemical methods and S35 uptake in the ground substance of the stellate reticulum, of the dental papilla, and of the dental pulp. In predentine, dentinal matrix, enamel matrix and dentinal tubules the staining methods used by us gave variable or negative results, and only occasionally was demineralization associated with a definite metachromasia. In the case of the dentinal tubules it was not possible to localize accurately the S35 uptake pattern.

Since chondroitin sulphate has been extracted from dentine, and since the S35 uptake pattern in the predentine and dentinal matrix was so constant in numerous sections, it is suggested that the isotope distribution is a more sensitive and reliable index of ester sulphates than metachromasia or other histochemical methods.

The composition of these sulphated materials cannot, of course, be decided without further, more direct evidence. However, metachromasia is not shown by sulphated esters of low molecular weight, and since intracellular metachromatic material was only rarely and weakly demonstrable in our sections, and since Bevelander & Johnson (1955) concluded that acid mucopolysaccharides, as shown by metachromasia, are confined to extracellular sites in developing dental tissues, it may be argued that, because intracellular S35 uptake has been clearly demonstrated, the complete polysaccharide is not synthesized in the cell.

Mode of uptake of S35. The mode of uptake of the inorganic S35 by the tissues cannot be determined with certainty in this investigation. Non-enzymic exchange of inorganic sulphate with ester sulphate is not significant, however, and enzyme-catalysed exchange of sulphate with preformed polysaccharide sulphate is now considered doubtful, because attempts to find such an enzyme in mammalian tissue have consistently failed. Thus the uptake of S35 as sulphate is generally accepted as evidence for polysaccharide synthesis. If it is postulated that hexosamines are esterified prior to the formation of polysaccharide then the lack of exact correspondence between the autoradiographic pattern and the pattern of metachromasia is explicable. The autoradiograph localizes even the early stages of the synthesis, but metachromasia is not shown by the sulphated esters of low molecular weight. The synthesis of sulphated polysaccharides starts, but is not necessarily completed, in the relevant cells. Bélanger (1954, 1955) confirmed the appearance of radioactive sulphate in the ameloblasts and suggested that only the ameloblasts of large type are capable of S35 synthesis and secretion. He gave no indication of whether radioactivity was observed in the odontoblasts or not. Leblond *et al.* (1955) favoured an extracellular sulphation hypothesis for dentinal mucopolysaccharide, and suggested that the bulk of the chondroitin sulphate of dentine is synthesized, or at least sulphated, at the predentino-dentinal junction. No evidence is given of the part played by the odontoblasts, if any. We have no evidence to confirm the idea of extracellular sulphation, and the distribution pattern of S35 in predentine did not show any marked increase at the predentino-dentinal junction. Our observations support the theory of cellular synthesis outlined above. In the case of the adult dental tissues the lack of definite activity in the odontoblasts and ameloblasts may be an

index of a small turn-over of sulphate as compared with the turn-over in the dental tissues of the younger animals.

The focal activity over cells of the stellate reticulum and of the dental pulp, and the more diffuse activity over cells and matrices, may be interpreted, as by Curran & Kennedy (1955*b*), as indicating the utilization of sulphate ion by fibroblasts in the production of the ground substance.

Enamel. With regard to amelogenesis, the present work suggests that cellular activity in the stratum intermedium and in the ameloblasts is associated with the appearance of a labelled sulphated mucopolysaccharide in the enamel matrix, and supports the view of Marsland (1951) that the ameloblasts and stratum intermedium form a functional unit concerned with the formation of matrix. Marsland (1951, 1952) also claimed that there is a primary phase during which the enamel organ is concerned solely with the deposition of an enamel matrix, which, although it contains a certain proportion of calcium salts, is insoluble in acids. The matrix formation is incremental in character, but maturation begins only after the full width of the matrix has been laid down, and occurs rapidly throughout the full thickness of the tissue in a plane at right angles to the axis of the tooth. We have no definite evidence of an incremental increase in the enamel matrix mucopolysaccharide component, but it may be that our usual picture of a uniform band of radioactivity occupying the full width of the matrix is associated with maturation. Between 164 and 169 hr. after injection a slight decrease in intensity was noted and, although no later observations were made, this decrease corresponds with the observations of Leblond *et al.* (1955) that mineralization may be associated with metabolic transformation leading to a loss of sulphur-labelled compounds. They also suggested that the matrix is dispersed by the accumulation of mineral elements to explain a diffuse, broad uptake pattern obtained in enamel, 2 days after injection of S35.

Dentine. Cellular activity in the odontoblasts may also be related to the appearance of the bands of radioactivity in the predentine and in the dentinal matrix. And the movement with time of the dentinal bands of activity may be explained by appositional growth of dentine, with incremental maturation. The dentine in the rat is laid down in daily layers about 16μ thick, and this rate of recession of the odontoblasts agrees quite well with the displacement of the bands of radioactivity. In the case of the double zoning in the dentinal matrix, on the convex side of an incisor, a possible explanation may be the relatively greater rate of growth on this side of the tooth. There may be a cyclical utilization of sulphate corresponding to incremental maturation and, where the growth rate *is a maximum*, a second cycle is visualized before the blood level of radioactive S35 has fallen. Thus in dentine, as in the enamel matrix, the sulphomucopolysaccharides appear to play a part in matrix formation and in maturation. Leblond *el al.* (1955) suggested that in the region of the predentino-dentinal junction a sulphated mucopolysaccharide, and probably another carbohydrate, are added to the collagen base of predentine, and concomitantly calcium and phosphate ions are deposited as dentinal crystals. Our observations fail to localize the main initial activity at the predentino-dentinal junction, but give a more diffuse uptake throughout the predentine. There is also an increase in intensity of the dentinal radioactivity up to 135–139 hr. after injection with a decrease in the last period observed.

Calcification. Sobel (1955), in an evaluation of the literature, pointed out that sulphate-containing mucopolysaccharides appear wherever calcification takes place, namely in dentine and enamel, in bone, and in abnormal calcification of the arteries. He suggested also that, for dentine and bone, chondroitin sulphate may be an integral part of the 'local factor(s)'. Lacroix (1954) postulated a role for S35 compounds in the formation of new *osteons* in bone, and Dziewiatkowski (1952) described a concentration of a sulphur-containing compound, or compounds, in centres of secondary ossification in the bones of young rats. In a later paper Dziewiatkowski (1954) found an increased accumulation of a sulphur-containing compound, in regions of active calcification, when vitamin A was administered to rats deficient of this vitamin. It was shown by Engfeldt, Engström & Boström (1954) that S35 was taken up in bone in the same sites as uptake of Ca45. Rubin & Howard (1950) put forward some evidence that a change in the state, or concentration, of chondroitin sulphate in cartilage matrix leads directly to the interaction of this compound with calcium, and thereby confers the state of calcifiability on the matrix. On the other hand, Sognnaes (1955) has suggested that sulphated mucopolysaccharides, rather than being the 'local factor', may serve to maintain certain regions in the uncalcified state, and provide metabolic pathways through relatively avascular and acellular structures. We have found that S35-labelled polysaccharides are distributed in dentine in a pattern corresponding with incremental maturation, and in enamel in a pattern corresponding with maturation *en bloc*. The final interpretation cannot yet be made on the facts available.

SUMMARY

1. S35 administered in inorganic form as sulphate is incorporated in organic form in the matrices of dentine and enamel, and in the ground substances of the dental pulp and stellate reticulum.

2. A cellular origin of the organic sulphated compounds, at least in part, is postulated, and evidence is presented for their synthesis in the odontoblasts, ameloblasts and cells of the dental pulp and stellate reticulum. There is no evidence of utilization of inorganic sulphate by the sulphur-containing amino-acids. The sulphated compounds are considered to be sulphomucopolysaccharides and their precursors.

3. The role of such compounds is discussed, and it is suggested that they may play a part in the mechanism of calcification of dentine and enamel.

The S35 was obtained from the Radiochemical Centre, Amersham. Our thanks are due to Prof. T. Symington and Dr A. R. Currie for their interest and encouragement, to Mr P. J. Elliot for the photography, and to the McGhie Cancer Research Fund for financial assistance.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Incisor in oblique coronal section: adult rat killed 19 hr. after injection. S35 is present in the predentine (*C*) as a uniform layer, and is present mainly focally in cells of the dental pulp (*A*). Odontoblasts (*B*) and dentinal matrix (*D*) are negative. The section does not include the enamel matrix. Coarse grain autoradiograph. ×220.

Fig. 2. Incisor in coronal section: foetal mouse killed 18 hr. after injection. S35 is present as a uniform band in the predentine (*C*); the enamel matrix (*E*), separated in processing from the ameloblasts (*F*), shows a lighter uptake. Fine grain autoradiograph. ×390.

Fig. 3. Molar in sagittal section: suckling rat killed 94 hr. after injection. The radioactive zone (*Z*) in the dentinal matrix is about equidistant from the predentine (*C*) and the amelo-dentinal junction (*J*) at the cusp. Fine grain autoradiograph. ×280.

Fig. 4. Molar in sagittal section: suckling rat killed 164 hr. after injection. The edge of the radioactive zone (*Z*) in the dentinal matrix (*D*) coincides with the amelo-dentinal junction. Fine grain autoradiograph. ×100.

Fig. 5. Incisor in sagittal section: suckling rat killed 118 hr. after injection. Two closely apposed zones of activity (*Y* and *Z*) are present in the dentinal matrix on the convex side of the tooth about one-third of the width of the matrix from the amelo-dentinal junction (*J*). A single zone (*X*) is present in the dentinal matrix on the concave side. The enamel matrix (*E*) shows a uniform uptake throughout its width. Fine grain autoradiograph. ×300.

Plate 2

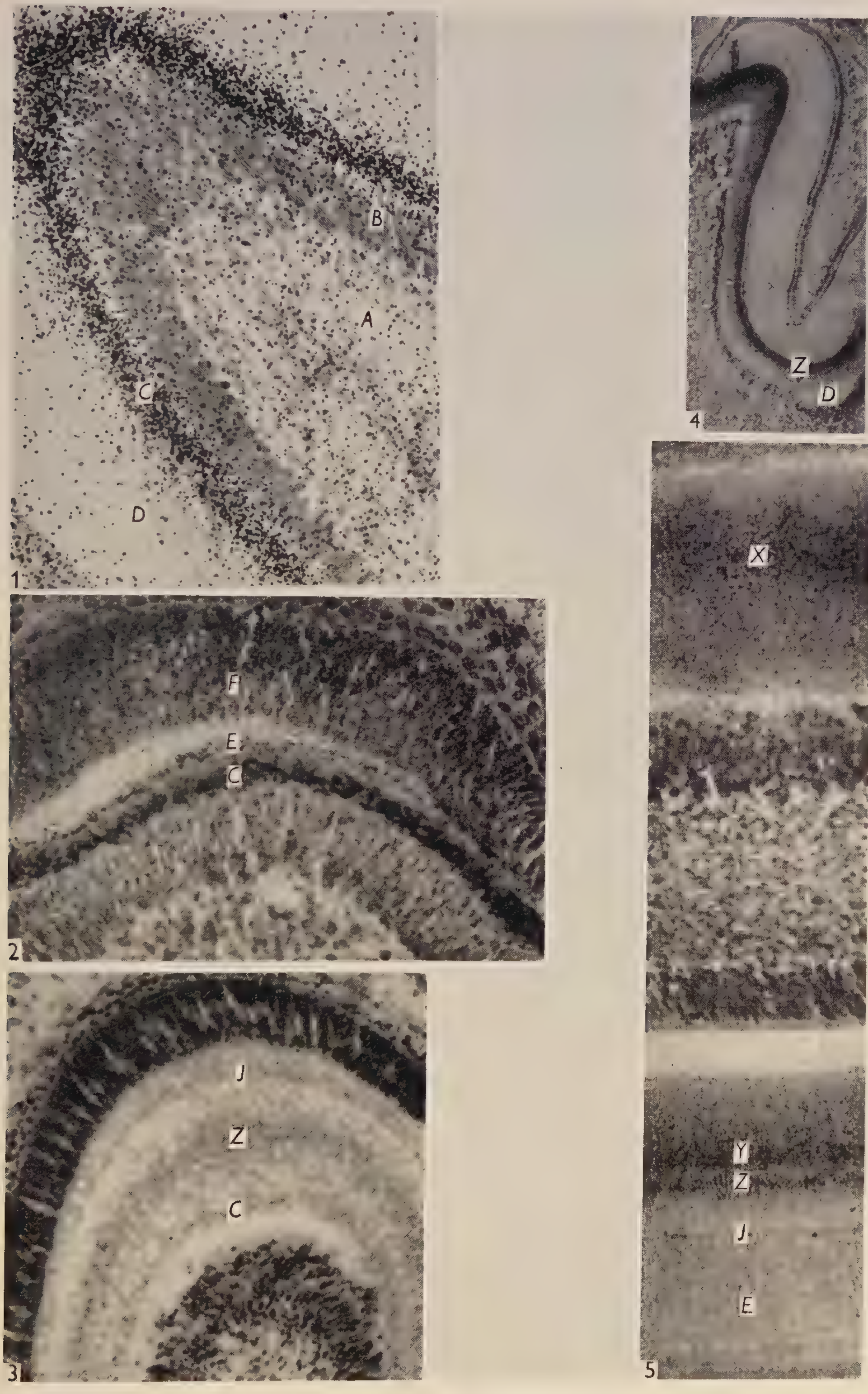
Fig. 6. Incisor in sagittal section: suckling rat killed 118 hr. after injection. The enamel matrix (*E*) shows a uniform uptake throughout its width. Cells of the dental pulp (*A*) show activity. Two zones of activity (*Y* and *Z*) are present in the dentinal matrix on the convex side of the tooth as in fig. 5. Fine grain autoradiograph. ×500.

Fig. 7. Molar in coronal section: suckling rat killed 8 hr. after injection. S35 is present in the odontoblasts (*B*), the ameloblasts (*F*), the stratum intermedium (*G*), the whole width of the enamel matrix (*E*), the predentine (*C*), and in some cells of the dental pulp (*A*). Fine grain autoradiograph. ×670.

Fig. 8. Incisor in coronal section: suckling mouse killed 16 hr. after injection. S35 is present in the ameloblasts (*F*) and in the stratum intermedium (*G*). Fine grain autoradiograph. ×1350.

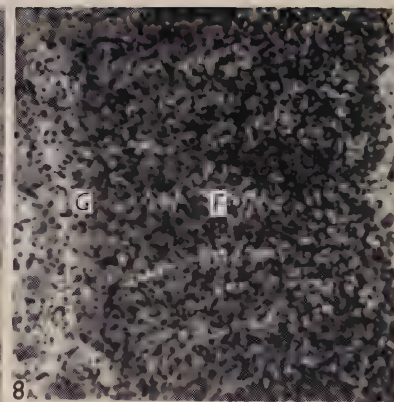
Fig. 9. Molar in sagittal section: suckling mouse killed 12 hr. after injection. S35 is present most intensely in the supranuclear parts of the ameloblasts (*F*), and in the stratum intermedium (*G*). Fine grain autoradiograph. ×1350.

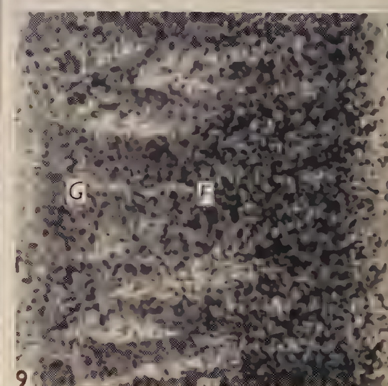
Fig. 10. Molar in coronal section: suckling rat killed 6 hr. after injection. S35 is present focally in cells of the stellate reticulum (*R*) and diffusely in the matrix. The stratum intermedium (*G*) and ameloblasts (*F*) also show an uptake. Fine grain autoradiograph. ×780.



KENNEDY AND KENNEDY—SULPHATED MUCOPOLYSACCHARIDES IN RODENT TEETH

(*Facing p.* 408)





KENNEDY AND KENNEDY—SULPHATED MUCOPOLYSACCHARIDES IN RODENT TEETH

A TECHNIQUE FOR THE ORIENTATION OF SERIAL HISTOLOGICAL SECTIONS

By W. R. BURSTON and K. THURLEY
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One of the fundamental requirements in any system of reconstruction involving the use of serial sections is some reliable system of orientation of the individual sections with respect of each other. Various devices have been employed, ranging from the selection of some natural structure in the specimen (e.g. the notochord of an embryo for use as a guide line), to the elegant but elaborate photographic technique of Heard (1931, 1951). Unquestionably this last technique represents the peak of development in reconstruction, but the elaborate and costly equipment required places it out of reach of normal investigations.

The method usually adopted is to incorporate orientating fibres into the paraffin block at the time of embedding of the specimen, and in this way obtain a series of marks surrounding each section. These marks can then be brought into register (provided certain requirements have been fulfilled—see below) as each successive outline is drawn on the projection apparatus.

The essential requirements of such a system are:

(1) That the fibres shall be parallel to each other and at right angles to the plane of section.

(2) That the fibres be of such a nature, that their 'sections' will adhere to the slide and not move in position throughout the subsequent staining procedures.

(3) That the technique be accurate within the limits imposed by the inherent errors introduced by section cutting.

Further desirable features are:

(1) That the system of 'markers' surrounding each section should fall within the field of the projection—that is the markers must be adjacent to the part studied.

(2) The method should be simple, convenient and cause as little interference with normal histological technique as possible.

With regard to the essential requirements, it is clearly necessary that the fibres be parallel to each other, otherwise there could be no possibility of accurate superimposition.

The demand that the fibres shall also be at right angles to the plane of section is perhaps not so obvious. Fig. 1*a* represents the condition in which the fibre is not at right angles to the plane of section.

Fig. 1*b* represents the distortion produced when the orientating fibre is subsequently used as a guide line. The correction of this distortion is difficult in a laminated three-dimensional model, even when the angle of error is approximately known. It involves sliding each plate into correct alignment so that the stack assumes a correct posture.

In case of graphic reconstruction, it will not be possible to calculate the angle of error with any certainty, since assumptions have to be made which vitiate the reconstruction.

The requirement that the fibre shall not move on the slide is obvious and demands that the fibre be of such a substance that it will take up the paraffin wax during the process of impregnation. In practice nerve fibres have been found to be most suitable for this purpose.

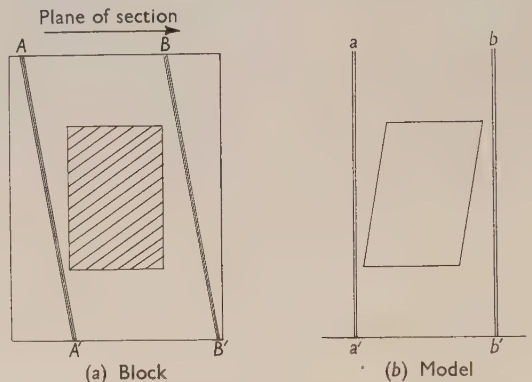

Fig. 1 (*a*). The shaded rectangle represents the specimen embedded in the paraffin block, as seen from the side. *AA'* and *BB'* are the nerve fibres which though parallel are not at right angles to the plane of section. In 1 (*b*) *aa'* and *bb'* are the guide lines corresponding to *AA'* and *BB'*, the resulting model being distorted as shown.

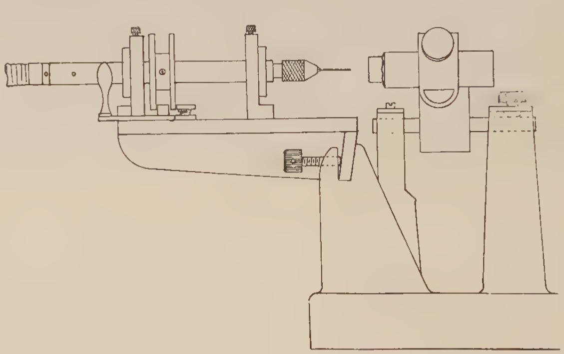

Fig. 2. Side elevation of the drill clamped in the knife holder of the flat-section cutting microtome. The drill is held accurately at right angles to the plane of section.

Among the many methods employed for the insertion of guide lines into the paraffin block may be mentioned those due to Peter (1906), Wilson (1910) and Pusey (1939).

Each of these methods has been tried in turn by the present writer, but each failed to meet one or other of the above requirements.

A technique was therefore evolved which consists of drilling the paraffin block accurately at right angles to the plane of section and then inserting a previously prepared nerve fibre into the hole, where it is secured by a soft wax lute.

The apparatus used consists of a simple, but very accurate drill, which clamps into the knife holder of the microtome (Fig. 2). The construction of the drill is such that

it can only rotate at right angles to the plane of section. The drill revolves to within $\frac{1}{1000}$ in. accuracy so that errors are minimal. It is so constructed that it can be moved across the whole face of the block.

A simple system of clamping is embodied on the arm of the microtome which can thus be held firmly in position during the process of drilling.

The drill is driven by a high-speed electric motor (3000 r.p.m.) via a flexible arm. This high speed is necessary in order to ensure accuracy when using very fine twist drills (see below).

The nerve fibre is prepared by tensing it across a stainless spring steel frame (Fig. 3). It is then stained, dehydrated, cleared and impregnated with normal embedding wax. The frame is removed from the molten wax, the excess wax is shaken off and the fibre allowed to set hard. It is then cut from the frame, care being taken not to bend it in any way or otherwise fracture the wax rod. The fibre is passed through progressively smaller holes in a draw plate (Fig. 3) so as to render it uniform by removing the excess wax. This process also allows the size of the fibre to be determined.

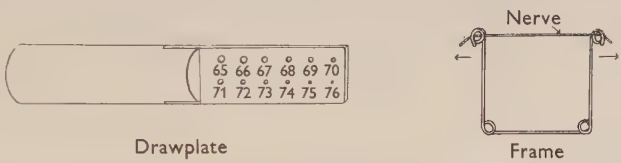

Fig. 3. The upper drawing represents the draw plate with graduated holes from 65–76 standard twist drill sizes. The lower drawing shows the spring frame for tensing the nerve fibres prior to impregnation with wax. The frame is made from 1 mm. hard stainless steel wire.

The fibres most commonly used range from 50 to 100 μ, which range is covered by standard twist drills nos. 60–80.

In practice the block is mounted on an orientating chuck-holder in the usual manner and the plane of section is established. The drill is then substituted for the knife and the holes are made. The appropriate nerve fibre (held between the finger and thumb to avoid bruising) is dipped into molten soft wax (at least 10° below the melting-point of the normal wax) and immediately pushed right home into the hole. The excess is trimmed off with a razor and the remaining fibres are similarly inserted.

Three points of reference are required in all cases—two are the minimum needed to locate a plane, the third serves as a check. Thus the minimum number of fibres required is two when using the midline as a check.

If the series is being cut specifically for a reconstruction four or even six fibres are desirable, but as a routine procedure two or three fibres suffice.

The nerve fibres may be inserted into the specimen where this is permissible or necessary for the study of some internal structure. Normally, however, they are placed in close proximity to the periphery of the specimen.

SUMMARY

A technique is described whereby guide lines may be readily incorporated into wax-embedded specimens prior to serial section. The method is accurate within the limits imposed by normal section techniques.

The authors wish to thank Prof. J. D. Boyd for his help and encouragement in developing the apparatus.

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REVIEWS

The Genesis of the Rat Skeleton. A Laboratory Atlas. By DONALD G. WALKER and ZOLTON T. WIRTSCHAFTER. (Pp. 59; $18 \times 12\frac{1}{4}$ in.; 57*s.* 6*d.*) Springfield, Illinois: Charles C. Thomas; Oxford: Blackwell Scientific Publications, Ltd.; Toronto: The Ryerson Press. 1957.

The authors claim to provide a pictorial record of osteogenesis in laboratory rats which will not only be a useful source of information for workers in this field, but will also afford them aesthetic satisfaction. In neither aim have they been wholly successful. Much of their work is repetitious, since the chronology of ossification in the rat has long been known from the work of Strong (1925), and is readily available in tabular form in Donaldson's *The Rat* (2nd ed. 1924). The times of appearance of certain sesamoid bones and of the epiphyses of the phalanges and caudal vertebrae are the only new items added; to be set against the omission of, for instance, the diaphysis of the humerus and the orbitosphenoid. No distinction is made in the tables—where, incidentally, a page has been misplaced, destroying the alphabetical order—between the periotic bone and the tympanic bulla, although the former ossifies well before the latter, as indeed can be seen in the subsequent illustrations. The structure labelled 'tympanic bulla' on p. 33 in a prenatal specimen is in fact not the bulla but the ectotympanic. In many instances the dates given do not coincide exactly with those obtained by Strong. This may be due to the different techniques used; Strong worked on unstained cleared material, and may, therefore, have at times mistaken calcified cartilage for bone. Alternatively, the discrepancies may result from the use of the Long–Evans strain of hybrid rats in the present investigation. The authors do not, however, state whether their figures represent mean values, how many animals were examined at each stage of development, or what variations, if any, were found to occur.

With so lavish a format, it seems pertinent to comment on the poor quality of the photographs, as they appear in reproduction. The frontispiece of the book, showing a mounted adult skeleton, is just adequate to indicate posture but retains no features of individual bones, the skull of the animal being particularly badly served. The remaining illustrations are of radiographs and alizarin-stained specimens, which are not always clearly distinguished one from the other by captions. Most of the alizarin preparations record the extent of ossification sufficiently well, but by comparison the X-rays, perhaps as a result of too long a development time for this type of work, are so lacking in detail and balance as to lead any reader to suppose that radiography is a useless tool in the study of osteogenesis. Such a conclusion would not be a true one; even at early stages of development, far better results can be obtained in the rat than are reproduced here.

C. C. D. SHUTE

Lymphatics, Lymph and Lymphoid Tissue. By J. M. YOFFEY and F. C. COURTICE. (2nd ed.; 60*s.*) London: Edward Arnold Ltd. 1956.

The publication of a book on the lymphatic system and lymph is in itself a sufficiently rare event to merit notice. The first edition of this book was written by Dr Drinker and Prof. Yoffey, and published in 1941 as one of the Harvard Monographs in Medicine and Public Health. In the second edition Prof. Yoffey is joined by Dr Courtice, who is himself a well-recognized authority in this field and already well known for his work on absorption from the serous cavities and his studies on the exchange of substances between the blood, tissue fluid and lymph.

This edition is considerably larger than its predecessor and, printed on art paper, is a much more pleasing production. The number of figures, including diagrams of lymphatic

pathways, graphs and tables is nearly doubled. There has been, in addition, some rearrangement of the subject-matter and large parts have been entirely rewritten. The references are full and up-to-date and now appear at the end of each chapter instead of at the end of the book as previously. Most readers will find this a great improvement. Space has been found for a somewhat more extensive treatment of the anatomy and development of the lymphatic system. It is still surprising how little has been added to the comparative anatomy of the system since the time of William Hewson, now nearly 200 years ago. It is equally surprising how little has been added to our knowledge of the distribution of the lymphatic pathways and glands in man since Mascagni, Cruickshank, Teichmann and Sappey. Likewise this publication reveals our scant knowledge of the permeability of the lymph capillary which is dealt with in the space of three pages. Asellus imagined his 'vasa lactea' to be open towards the intestine, and workers such as the Hunters, Cruickshank and Sheldon, who did so much to establish the functions of the absorbent vessels, were not faced with great difficulties in explaining the route of entry of substances into the vessels. In the last century, however, much energy was devoted to the study of the capillary wall and of the so-called stomata in the lymphatic capillaries. Here we are reminded of such names as von Recklinghausen, Ludwig, Tomsa, Frey, Teichmann and many others. This problem settled, however, little has been done to elucidate the problem of the permeability of the lymphatic capillary as compared with that of the blood capillary. This must not be interpreted as a criticism but rather as a recommendation of the book, a brief perusal of which reveals many other problems of equal importance which await elucidation. It is essentially a book for the research worker and should be readily available to anatomists, physiologists, biochemists, pathologists and clinicians alike. The topics dealt with are of first importance in clinical medicine and deserve much more attention than they have received in the past. D. V. DAVIES

Ciba Foundation Colloquia upon Ageing. Volume 2. *Ageing in Transient Tissues.* (Pp. xi+263; 96 illustrations; 36*s.*) J. and A. Churchill Ltd. 1956.

The papers presented at this colloquium covered topics ranging from the physiology of antler regeneration to the life-span of red corpuscles. The unity of thought behind the treatment of these subjects suffered considerably from divergences of intention, transient tissues being taken to include those which are continuously or cyclically renewed, as well as those which, like the placenta or the tadpole's tail, degenerate developmentally. The most interesting papers, those of Price and Pannebecker and of Jøst on foetal organ culture in the presence of hormones, have no immediate relevance to 'ageing' in its wider sense, and only the papers of Lovelock and of Mollinson, on the nature of the factors which limit red cell survival, approach the fundamental question of the processes which limit the life of non-dividing cells. The evidence against the renewal of mammalian oocytes in postnatal life is once again presented by Zuckerman, and there are interesting accounts by Montagna of the regression of the axillary sweat glands with age and by Tuchmann-Duplessis and Mercier-Parot on the action of pituitary somatotrophin injected into the mother upon the foetal rate of growth and development, both of which it retards. The remaining communications deal with the fate of redundant follicles (P. C. Williams), the corpus luteum of the guinea-pig (I. W. Rowlands), the cytomorphosis of human testicular interstitial cells (D. W. Fawcett and M. H. Burgos), mitochondrial changes during cell activity (E. W. Dempsey), morphology (G. B. Wislocki), biochemistry (C. A. Villee) and function (A. G. Huggett) of the placenta in relation to its age, ^{42}K uptake during the course of pregnancy (R. J. Harrison and J. L. D'Silva), the growth-cycle of antlers (G. B. Wislocki) and the senescence of leaves (E. W. Yemm). Apart from one or two which are too briefly reported to be explicit, all these papers are of interest, though not primarily, perhaps, to the student of ageing in whole organisms. The discussion is fully reported. ALEX COMFORT

A Manual of Human Anatomy. By J. T. AITKEN, G. CAUSEY, J. JOSEPH and J. Z. YOUNG. Vol. I: *Thorax and Upper Limb*, 162 pp., 36 figs., 14*s.* Vol. II: *Head and Neck*, 180 pp., 53 figs., 16*s.* Vol. III: *Abdomen and Pelvis*, 135 pp., 29 figs., 12*s.* 6*d.* Vol. IV: *Lower Limb*, 117 pp., 36 figs., 12*s.* 6*d.* Vol. V: *Central Nervous System*, 140 pp., 40 figs., 12*s.* 6*d.* Edinburgh and London: E. and S. Livingstone Ltd.

No one engaged on teaching medical students can fail to be aware of the growing burden which candidates for the second medical examination seem called upon to bear; and the advent of a new manual of human anatomy which might serve to lessen this load, and yet be a sound guide to the student dissecting the body for the first time, would be indeed a blessing. To the reviewer's mind this was the task set themselves by the authors, and it is his responsibility to state how far, in his view, they have succeeded.

The general plan of the work is that dissection should commence with the thorax and thereafter proceed to the upper limb, head and neck, abdomen and pelvis, and lower limb. There is much in favour of such a sequence, but for schools in which a different programme is followed alternative instructions are given. For the most part these should prevent any difficulty, but in certain circumstances, for example when dissection of the lower limb is begun on the front of the thigh, further guidance will be needed. Two of the steps taken by the authors will, in principle, meet little opposition; the omission of detail, which has been pleaded for over many years, and the consideration of the functional implications of the structural arrangement of parts. To get general agreement on what constitutes detail is probably impossible, and consequently it may be thought fruitless to instance omissions from the present manual as being either right or wrong. Welcome features of each volume are the end chapters in which are summarized the cutaneous nerve supply, the lymphatic drainage, and those points in the osteology and surface anatomy of the part with which students should become familiar. These last are commonly studied in special small classes, and junior demonstrators as well as undergraduates will find the suggestions which are made very helpful.

The figures throughout the volumes are in line, with, however, a fair use of colour. They are designed mainly to illustrate the text and, many being somewhat schematic, will not encourage 'armchair' anatomy. However, overall, there do not seem to be sufficient. For example, the hand seems very sparsely treated in this regard, and in Vol. II a figure of a sagittal section through the head and neck would seem almost essential for an understanding of the pharynx.

Vol. V which deals with the Central Nervous System is written by J. T. Aitken, J. Z. Young and D. A. Sholl, and is of a more detailed character than the others. The authors state that some teachers may consider its content to be in excess of what is needed for the second medical examination but these will surely be in the minority. Besides treating of those features of the brain, its membranes and blood supply which can be studied macroscopically in the whole brain and in the sectioned or dissected specimen, considerable attention is paid to the features presented in neurohistological sections of the spinal cord and brain stem. The main fibre tracts are followed throughout their extent, and wherever possible the functional significance of their connexions is emphasized. But deep dissection of the cerebral hemisphere to show the corona radiata and internal capsule or the long association bundles is not mentioned. Such procedures are time-consuming, and in many cases unrewarding, but when successful give an understanding of cerebral anatomy which sections alone cannot.

It is in fact when the manual is judged as a guide to the dissection of the body that it appears least satisfactory. Admittedly the phrase 'dissection of the body' is open to wide interpretation, and herein lies the greatest difficulty facing the authors of any dissecting manual; but there is a danger in relaxing standards in this regard. Not to see the wood for the trees is certainly a bad thing but overenthusiastic tree-felling will just as surely bring about the same result. In the inguinal region, for example, it seems a pity that dissection

of the lymph nodes should not be undertaken. Nowhere else in the body can students see for themselves with so little trouble a series of regional nodes, furnished with easily dissectable afferent and efferent lymphatic vessels, whereby an appreciation of this important system can be gained. Again, to dissect the nutrient artery of the humerus or the articular nerves to the knee joint are not unworthy pursuits; indeed a student may gain just as vivid a conception of bone as a living tissue, or of the proprioceptive sense, by such simple methods of discovery as by others much more complex. However, whatever the standard of dissection expected it is essential that both the text and the figures which accompany it in a dissector's guide should possess a high degree of accuracy, for only then will the student gain the confidence necessary for success, and equally important, a true respect for the new discipline which is to serve as the basis for his future professional work.

Amongst the statements and illustrations which need emendation are the following:

The use of the word fascia when aponeurosis or ligament is intended, e.g. the origin of the pectoralis major from the *fascia* of the anterior abdominal wall and that of the rectus abdominis from the *fascia* in front of the pubis; the statement that the lower seven (intercostal) nerves end in the anterior abdominal wall; the inclusion of the geniohyoid as an extrinsic muscle of the tongue, and of the external jugular lymph nodes in the deep cervical group; the dimensions of the middle ear (6 mm. anteroposterior diameter is too small); one or other of the statements that the saphenous opening lies 3 cm. (Vol. IV, p. 16) and 4 cm. (p. 103) below and lateral to the pubic tubercle; the phrenic nerve/vagus nerve relationship in fig. 8 of Vol. I; the course of the circumflex nerve in fig. 16 of Vol. I; the size of the first palmar interosseous muscle in fig. 30B of Vol. I; the position of the infrahyoid muscles in fig. 11 of Vol. II; the position of the hypoglossal nerve in fig. 14 of Vol. II; the positions of the abducent nerve in fig. 32 of Vol. II; the statement that the small muscles of the foot flex the toes at the interphalangeal joints. Typographical errors, inescapable from any first edition, are few.

In summary it may be said that this book represents a point of view with regard to the whole purpose of dissection at undergraduate level and to the amount of information it is proper to expect of students. Anatomists, certainly in this country, will doubtless be divided for and against, but no one can gainsay the authors' courage in taking the stand they have or their industry in preparing this work. The reviewer has found his task of considerable interest but finds that he is as yet unable completely to ally himself with this new approach to the dissection of the human body.

E. W. WALLS

BOOKS RECEIVED

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A QUANTITATIVE STUDY OF THE FORNIX-MAMILLO-THALAMIC SYSTEM

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INTRODUCTION

Recent studies of the fornix-mamillo-thalamic system have drawn attention to the relative sizes of some of the parts of this system and to some striking interspecies differences. Simpson (1952) and Daitz (1953) counted the fornix fibres in the monkey and in man and showed that only one-quarter to one-third of the subcallosal fibres reach the level of the mamillary bodies. This suggests that the pre-commissural, septal, part of the fornix forms the greater portion of the fornix system (e.g. Bucy & Klüver, 1955) but at present it is difficult to give an estimate of the size of the pre-commissural fornix, since Guillery (1955) showed that between one-third and one-half of the post-commissural fibres of the rabbit and the cat fail to reach the mamillary bodies. The majority of these non-mamillary fibres in the post-commissural fornix probably pass to the anterior thalamus; Nauta (1956) and Guillery (1956) have found a considerable projection from the fornix to the anterior thalamus in the rat.

Guillery (1955) also showed that the number of hippocampo-mamillary fibres is approximately equal to the number of cells in the medial mamillary nucleus and to the number of fibres in the mamillo-thalamic tract. This relationship and the precise organization of the mamillo-thalamic projection (Powell & Cowan, 1954) suggested that the extension of the quantitative studies to the anterior thalamic nuclei might throw further light on the organization of this system.

In the present investigation counts were made of the fibres in the post-commissural fornix at varying levels along its course, of the cells in the medial mamillary nucleus, of the fibres in the mamillo-thalamic tract and of the cells in each of the anterior thalamic nuclei. The brains of rats, rabbits, cats, monkeys and man have been studied.

MATERIALS AND METHODS

The cells and fibres have not been counted in the same brain since the histological preparation and the plane of section were different for each group of counts.

Cell counts

The cells in the medial mamillary nucleus and in the three anterior thalamic nuclei were counted in at least three brains of each species (excluding man). Only one human brain was used, complete counts being made in each hemisphere. All the brains were fixed in 70 % alcohol and 2 % acetic acid, embedded in paraffin and sectioned coronally at 15–25 μ. In the rabbit, cat and monkey (*Macaca mulatta*) every fifth section was mounted. In the human brain every tenth section was mounted and in the rat alternate sections were usually taken. All the sections were

stained with a 1 % solution of thionine. The total number of cells in a nucleus was calculated from (*a*) the total volume of the nucleus, and (*b*) the mean number of cells in a unit volume (the cell density). The volumes were measured by the method of Dornfeld, Slater & Scheffe (1942). The cell density was estimated from a series of sample cell counts using a field of known diameter under an oil immersion objective. The number of these fields was approximately proportional to the cross-sectional area of the nuclear group at each level of its antero-posterior extent. As a rule between 2 and 5 % of the total number of cells in a nucleus were counted. No correction factor has been used in calculating the totals, since only cells with a distinct nucleolus have been counted. Each of the final figures represents the mean of two independent counts. Where these differed by more than 20 % a third count was made.

Fibre counts

The fibres in the post-commissural fornix and mamillo-thalamic tract have been counted in one human brain, two monkey brains, one rabbit, one cat and two rat brains. Serial sections were cut at 7–10 μ at a plane perpendicular to the post-commissural fornix and stained by the Holmes silver method (1947).* The fornix counts were taken from a number of sections spaced along this series and the mamillo-thalamic counts were taken from the part of the series that passed perpendicular to the mamillo-thalamic tract. The fibres were counted under an oil-immersion objective in a square field of side 4·7 μ. Between 100 and 500 such fields, chosen at random, were counted in each section, the total number of fields counted being dependent upon the size of the tract. The coefficient of variation of the individual counts was usually between 10 and 15 %. It never exceeded 20 %.

The cross-sectional areas of the tracts were measured by counting the number of square millimetres occupied by a low-power photograph of the tract. This method has been compared with that used previously (Guillery, 1955) and it has been found that the results of the two methods differ by less than 5 %. The counts of the post-commissural fornix and the mamillo-thalamic tract of the rabbit and the cat given by Guillery (1955) have been included in Tables 1, 2 and 4, but in one animal of each of these series an additional count has been made in the fornix rostral to the level of the medial cortico-habenular tract.

The post-commissural fornix

Table 1 shows the total number of fibres in the post-commissural fornix at varying levels along its course, and gives the levels from which each count was taken. None of the counts was taken from a level anterior to the anterior commissure. At the levels shown by the negative figures in Table 1 the fornix lies dorsal to the anterior commissure and its dorsal aspect is clear of the septum, so that none of the fibres of the pre-commissural fornix has been included in any of the counts. The position of the 'medial cortico-habenular' tract is shown in the last column of the table. The medial cortico-habenular tract is here regarded as the bundle that can be seen on normal material running between the fornix and the stria medullaris. The name is retained although the bundle appears to consist of septo-habenular and hippocampo-

* In stage 2 of the procedure 3 ml. of 1 % aqueous silver nitrate and 5 ml. of pyridine were used. In stage 6 the reducer was heated to 50° C.

Table 1. *The number of fibres in the post-commissural fornix at various levels along its course*

Distance behind the anterior commissure expressed as a percentage of the distance between the commissure and the mamillary bodies (%)	No. of fibres in post-commissural fornix	Position of the medial cortico-habenular tract	Distance behind the anterior commissure expressed as a percentage of the distance between the commissure and the mamillary bodies (%)	No. of fibres in post-commissural fornix	Position of the medial cortico-habenular tract
	Rat 222			Cat 4*	
−9	51,000	From −7	1	184,000	Anterior to 1 %
0	38,000	to 2 %	40	127,000	
35	29,000		61	114,000	
70	18,000		99	138,000	
100	17,000				
	Rat 240			Cat 16	
−13	66,000	From −10	−6	256,000	From −4
−9	56,000	to 1 %	4	176,000	to 2 %
11	38,000		70	97,000	
72	22,000				
100	22,000				
	Rabbit 3*a**			Monkey 01	
−15†	236,000	From −6	−7	185,000	Anterior to −7 %
4	203,000	to 1 %	16	128,000	
10	168,000		49	105,000	
23	123,000		75	82,000	
36	123,000		100	81,000	
49	96,000				
73	92,000				
95	103,000				
103	95,000				
	Rabbit 4*			Monkey 02	
5	190,000	Anterior	−37	220,000	−25 to 12 %
8	145,000	to 5 %	−10	222,000	
30	122,000		2	126,000	
52	99,000		31	102,000	
95	90,000		63	97,000	
			100	103,000	
	Rabbit 66			Man (R)	
−11	202,000	From −7	−44	1,245,000	Approx. from
0	160,000	to 0 %	−33	1,310,000	−33 to −6 %
84	64,000		−6	1,321,000	
			14	959,000	
			35	1,058,000	
			56	892,000	
			89	802,000	
	Cat 3*a**			Man (L)	
−17†	267,000	From −11	−42	1,220,000	Approx. from
2	143,000	to −2 %	−27	1,230,000	−33 to −6 %
15	131,000		−7	1,323,000	
15	142,000		21	956,000	
29	89,000		47	1,002,000	
45	78,000		72	768,000	
60	90,000		98	636,000	
−60	96,000				
88	102,000				

* These counts are taken from Guillery (1955)

† These counts were not included in the previous series (Guillery, 1955).

thalamic fibres, and to contain no fibres that pass from the hippocampus to the habenula (Sprague & Meyer, 1950; Powell & Cowan, 1955; Guillery, 1956; Nauta, 1956).

The fornix counts of the rabbit, cat and monkey are remarkably similar, both at the level of the medial cortico-habenular tract and at the pre-mamillary level. At the rostral level there are about 220,000 fibres in the monkey, about 260,000 fibres in the cat and just over 200,000 fibres in the rabbit. At the level of the mamillary nuclei the values are: monkey 80,000, cat 100,000–140,000 and rabbit about 100,000. The corresponding values for the rat are about one-fifth as great and those for man approximately five times as great. The table also shows considerable individual variation in fornix size in any one species, as seen in cats 3 and 4 or in the rabbit counts.

Table 2. *The total fibre loss from the post-commissural fornix*

	Number of fibres in fornix column immediately anterior to the medial cortico-habenular tract	Number of fibres immediately rostral to the mamillary nuclei	Total percentage fibre loss between these two levels (%)
Rat 222	51,000	17,000	67
240	66,000	22,000	67
Rabbit 3*a*	236,000	95,000	60
66	202,000	64,000	68
Cat 3*a*	267,000	102,000	62 (71)*
16	256,000	97,000	62
Monkey 02	220,000	103,000	53
Man (L)	1,220,000	636,000	48
(R)	1,310,000	802,000	39

* In this estimate the lowest figure for the post-commissural fornix has been used (78,000—about half-way between the anterior commissure and the mamillary nuclei).

In Table 2 the number of fibres which leave the fornix during its course through the hypothalamus is expressed as a percentage of the number of fibres in the fornix immediately rostral to the medial cortico-habenular tract. Only the animals in which these rostral counts are available have been included, and in each case the most posterior, pre-mamillary count has been used for comparison. In the cat, however, the lowest values for the post-commissural fornix come from levels well anterior to the mamillary bodies and show a greater percentage loss in the hypothalamus, amounting to 71 % in cat 3*a*.

In each of the animals a large number of fibres leaves the hypothalamic part of the fornix. In the rabbit, rat and cat this fibre loss amounts to about two-thirds of the total number of fibres in the post-commissural fornix, while in the monkey it is just over one-half and in man just under one-half of the total. The total fibre loss in the rabbit and the cat is greater than that given by Guillery (1955), since the part of the fornix dorsal to the anterior commissure and rostral to the medial cortico-habenular tract has been included in the present counts. Figs. 1–5, drawn from the data in Table 1, show the pattern of this fibre loss in each of the species. In the rat approximately one-half of the loss occurs just rostral to the posterior border of the medial cortico-habenular tract and the remainder occurs more gradually posterior

to the tract. In the rabbit the loss opposite the medial cortico-habenular tract is relatively small, while the sharpest loss occurs immediately behind this tract and most of the fibres leave the fornix in the rostral third of the hypothalamus. The fibre loss in the cat resembles that in the rat, but in addition, in the two animals in which

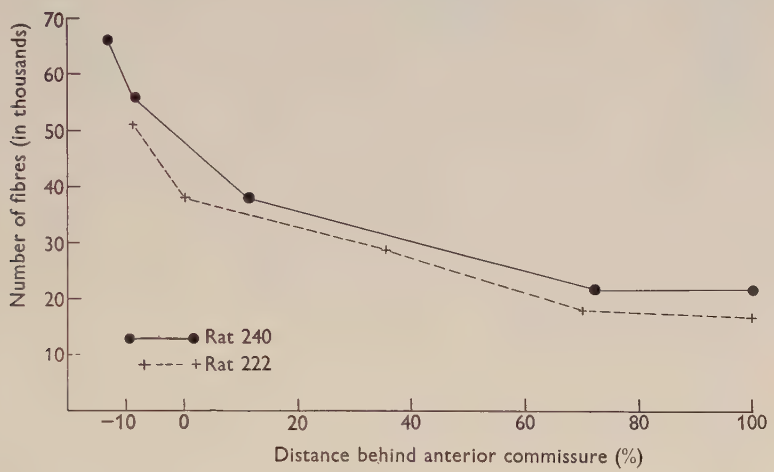

Fig. 1. Graph showing the pattern of fibre loss from the post-commissural fornix in the rat. This and the subsequent graphs have been drawn from the data in Table 1. The position of the medial cortico-habenular tract in each brain is shown by the horizontal line in the lower left-hand corner.

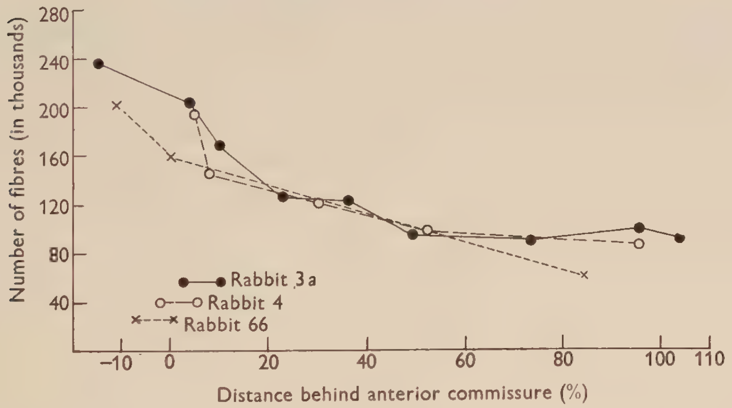

Fig. 2. Graph showing the pattern of fibre loss from the post-commissural fornix in the rabbit.

the relevant counts have been made (cats 3 and 4), there is a secondary rise in the caudal half of the hypothalamus which amounts to about 24,000 fibres. The monkey and human counts show no evidence of a loss opposite the medial cortico-habenular tract; in the monkey there is a fairly sharp loss behind the tract and in man there is a gradual loss which appears to extend through the whole length of the hypothalamus.

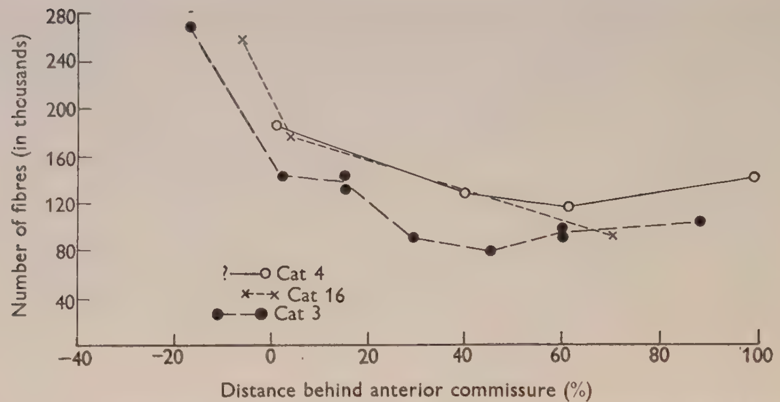

Fig. 3. Graph showing the pattern of fibre loss from the post-commissural fornix in the cat.

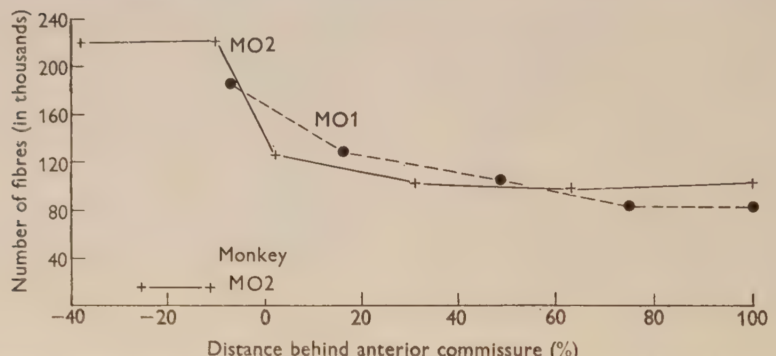

Fig. 4. Graph showing the pattern of fibre loss from the post-commissural fornix in the monkey.

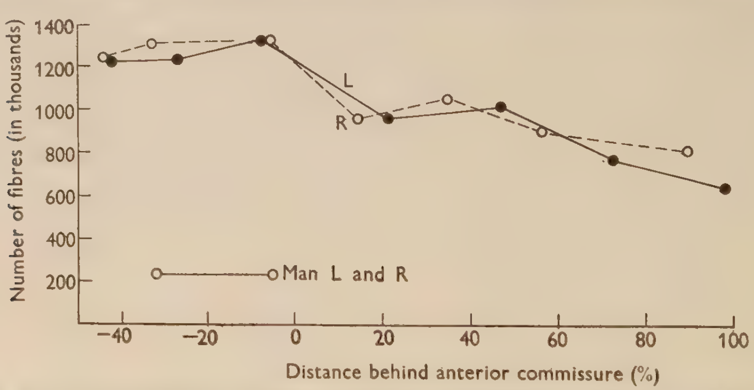

Fig. 5. Graph showing the pattern of fibre loss from the post-commissural fornix in the human brain.

The medial mamillary nucleus

In Table 3 the total number of cells in the medial mamillary nucleus (proper) is given. The counts do not include the cells in the supra- and pre-mamillary groups, nor has any estimate of the number of cells in the different subdivisions of the medial mamillary nucleus been made (see below).

The counts for the rabbit, the cat and the monkey are again strikingly similar although the values for the rabbit are higher than the others. In the rat there are about half as many cells as in the cat and the monkey, while in the human brain there are about five times as many cells.

Table 3. *The number of cells in the medial mamillary nucleus*

Rat CB2	32,000	Monkey M12	84,000
EB3	45,000	M3	71,000
RF9	35,000	M2	68,000
RC1	37,000	M5	74,000
Rabbit 43	120,000	Man R	385,000
40	116,000	L	393,000
	104,000*		
	130,000*		
Cat 10	78,000		
9	81,000		
CG1	79,000		
	84,000*		
	74,000*		

* These counts are taken from Guillery (1955).

The pre-mamillary fornix and the medial mamillary nucleus

Comparison of the number of fornix fibres immediately anterior to the mamillary bodies with the number of cells in the medial mamillary nucleus shows a fibre/cell ratio of the order of 1:2 in the rat, 1:1 in the rabbit, cat and monkey and 2:1 in man. Since the secondary increase in the pre-mamillary fornix of the cat must be due either to the branching of individual fornix fibres or to hypothalamic fibres which join the posterior part of the fornix, the lowest value for the fornix counts in the cat has been used in preference to the pre-mamillary value.

In interpreting these ratios the interspecies differences that have been found in the mamillary distribution of the fornix terminals have to be considered (Sprague & Meyer, 1950; Simpson, 1952; Guillery, 1956). Thus, with the possible exception of the primate brain, not all the cells of the medial mamillary nucleus receive afferents from the hippocampus and different subdivisions of the nucleus may not receive the same relative number of fornix fibres; conversely, not all the fibres of the pre-mamillary fornix terminate in the medial mamillary nucleus, as there are some hippocampo-mesencephalic fibres. It must also be emphasized that an overall ratio of 1:1 does not imply that one cell receives only one fornix fibre. However, there appears to be a relationship between the cell/fibre ratios and the cell density in the medial mamillary nucleus. Thus, the cell density per cubic mm. is approximately 110,000 in the rat, between 30,000 and 60,000 in the rabbit, cat and monkey and about 15,000 in man.

The mamillo-thalamic tract

The number of fibres in the mamillo-thalamic tract of each of the five species is shown in Table 4. These values are again similar in the rabbit, cat and monkey and markedly different in the rat and in man.

The variations in any one group of counts (amounting to as much as 30 % in the cat) are probably due to two factors. The outline of the mamillo-thalamic tract is not as sharp as that of the fornix, particularly in the cat and in man, where an additional error may thus have been introduced into the measurement of the cross-

sectional area. Differences in the levels at which individual counts were made may also account for a part of the variation, as there is some evidence to suggest that the individual fibres of the mamillo-thalamic tract branch in their course between the mamillary bodies and the anterior thalamus. For these reasons the different values found in the rabbit, cat and monkey may not be as significant as they appear at first sight.

Since the values of the mamillo-thalamic counts are close to the values given for the pre-mamillary fornix the ratios of mamillo-thalamic fibres to medial mamillary cells would be close to the ratios given in the preceding section.

Table 4. *The number of fibres in the mamillo-thalamic tract*

Rat	240	25,000	Cat		115,000*
		28,000			72,000*
Rabbit	222	73,000*	Monkey	01	92,000
		82,000*		02	113,000
		Man	572,000		L
			507,000		L
			604,000		R
			489,000		R

* These counts are taken from Guillery (1955).

The anterior thalamic nuclei

The cell counts of the three anterior thalamic nuclei are given in Table 5, together with the total anterior thalamic count in each brain. Comparison of totals shows exactly the same relationship as has been found in all the previous comparisons, that is, the values for cat, rabbit and monkey are closely similar, those for the rat much smaller and those for man much greater.

Table 5. *The number of cells in the three anterior thalamic nuclei*

		Anteroventral nucleus	Anteromedial nucleus	Anterodorsal nucleus	Total
Rat	CB2	37,000 (63)	12,000 (20)	10,000 (17)	59,000
	EB3	41,000 (61)	14,000 (21)	12,000 (18)	67,000
	RF9	42,000 (64)	16,000 (24)	8,000 (12)	66,000
Rabbit	43	75,000 (48)	39,000 (25)	41,000 (27)	155,000
	45	77,000 (59)	32,000 (25)	21,000 (16)	130,000
	36	73,000 (51)	39,000 (28)	30,000 (21)	142,000
Cat	9	72,000 (51)	50,000 (36)	18,000 (13)	140,000
	CG1	54,000 (43)	54,000 (43)	17,000 (14)	125,000
	10	61,000 (44)	54,000 (39)	25,000 (18)	140,000
Monkey	M12	62,000 (47)	50,000 (38)	20,000 (15)	132,000
	M 2	63,000 (52)	47,000 (38)	12,000 (10)	122,000
	M 5	64,000 (52)	40,000 (33)	19,000 (15)	123,000
Man	R	647,000		Not counted	647,000
	L	680,000			680,000

Figures in brackets give the percentage of the total anterior thalamic count.

The counts of the individual anterior thalamic nuclei show further interesting features. The counts of the anterodorsal nucleus in any one species, particularly in the rabbit and the monkey, show wide differences. These can only partly be due to the inaccuracy of cell counting in this nucleus, since repeated counts of the same individual nucleus (and by different observers) have consistently given essentially the same values. There may thus be more individual variation in this nucleus than

in the other anterior thalamic nuclei, but it is none the less apparent that the anterodorsal nucleus is larger, relatively and absolutely, in the rabbit than it is in the other species. Thus, in the rabbit about 21 % of the anterior thalamic cells are in the anterodorsal nucleus, compared with 16, 14 and 13 % in the rat, cat and monkey, respectively. This difference in the rabbit is almost certainly related to the unusually large extent of the retrosplenial cortex.

It has not been possible to count the cells in the anterodorsal nucleus of the available human material for although the nucleus can be readily identified, it is so small and its margins are so poorly defined that any attempt to estimate the number of cells would have been inaccurate and misleading. Qualitatively the material gives the impression that relative to the rest of the anterior thalamus the anterodorsal nucleus has undergone a marked reduction in man.

In Nissl-stained sections the precise boundary between the anteromedial and anteroventral nuclei is not always easy to define. It is generally agreed that this is particularly so in the human brain and since no clear delimitation could be found in the present human material the two nuclei have been considered together. In the rat and the rabbit the limit between the two nuclei was quite definite but in the cat and the monkey the boundary was not so well defined, so that there may be some error in the relative size of the two nuclei. However, this error is probably less than 10 % and does not prevent the following comparisons.

The size of the anteroventral nucleus relative to the anteromedial nucleus is greatest in the rat (3:1), less in the rabbit (2:1) and least in the cat and monkey (1:1). Relative to the total anterior thalamic count the rat anteroventral nucleus is again the largest, forming just under two-thirds of the total, while in the rabbit, the cat and the monkey it forms approximately half of the total. None of the counts supports the view that the anteroventral nucleus is relatively large in primates. The converse should also be emphasized; these counts show that the anteromedial nucleus is relatively larger in the cat and primate brain than in the rabbit and rat.

The volumes of nuclei and cell density

In Tables 6 and 7 the volume of each nucleus is given together with a figure for the mean cell density within that nucleus. As with the cell counts there is a certain variation in the volumes within any one species. However, in a number of instances, as in the anterior thalamic nuclei of the human brain and in rabbit 36, a marked difference in the volume is balanced by a compensatory difference in cell density. In other instances, as in the anterodorsal nuclei this is not so and there is a marked difference in the cell count. The variability of the volume measurements may be partly due to variable shrinkage effects during fixation and preparation but it is probable that other factors also play a part.

In spite of this variability a comparison between the species is possible, and it is clear that neither cell counts nor volumetric measurements by themselves can give a complete picture of the relative 'size' of any nucleus. Thus, the volume of the anteroventral nucleus of the rabbit is considerably less than that of the cat or the monkey but the mean cell count is lower in the last two species. Again, of these three species the medial mamillary nucleus of the cat has the greatest volume but the lowest cell density. It is interesting to compare this result with that found in the

Table 6. *The mean cell density* (i.e. *number of cells/mm.*³) *in the medial mamillary nucleus*

		Volume of nucleus (mm.³)	Total cell count	Cells/mm.³ (to nearest 1000)	*Total brain weight (gm.)
Rat	CB2	0·321	32,000	100,000	1·6
	RC1	0·327	37,000	113,000	
	RF9	0·318	35,000	110,000	
	EB3	0·340	45,000	132,000	
Rabbit	43	1·940	120,000	62,000	9·3
	40	1·951	116,000	60,000	
Cat	10	2·640	78,000	30,000	31
	CG1	2·635	79,000	30,000	
	9	3·072	81,000	26,000	
Monkey	M12	1·667	84,000	51,000	88·5
	M3	2·092	71,000	34,000	
	M2	2·146	68,000	32,000	
	M5	2·112	74,000	35,000	
Man	R	24·710	385,000	16,000	1320
	L	27·970	393,000	14,000	

* These figures are taken from Count (1947).

Table 7. *The mean cell density in the anterior thalamic nuclei*

		Anteroventral nucleus			Anteromedial nucleus			Anterodorsal nucleus		
		Volume of nucleus	Cell count	Cells/mm.³	Volume of nucleus	Cell count	Cells/mm.³	Volume of nucleus	Cell count	Cells/mm.³
Rat	CB2	0·388	37,000	95,000	0·201	12,000	60,000	0·086	10,000	116,000
	EB3	0·358	41,000	114,000	0·158	14,000	89,000	0·098	12,000	122,000
	RF9	0·420	42,000	100,000	0·262	16,000	61,000	0·076	8,000	105,000
Rabbit	43	1·881	75,000	40,000	1·222	39,000	32,000	0·512	41,000	80,000
	45	1·750	77,000	44,000	1·210	32,000	26,000	0·331	21,000	63,000
	36	1·326	73,000	55,000	0·779	39,000	50,000	0·396	30,000	76,000
Cat	9	3·710	72,000	19,000	2·410	50,000	21,000	0·751	18,000	24,000
	CG1	2·335	54,000	23,000	2·580	54,000	21,000	0·690	17,000	25,000
	10	2·429	61,000	25,000	2·230	54,000	24,000	0·865	25,000	30,000
Monkey	M12	4·320	62,000	14,000	3·580	50,000	14,000	0·612	20,000	32,000
	M2	4·391	63,000	14,000	3·250	47,000	14,000	0·370	12,000	32,000
	M5	5·160	64,000	12,000	3·116	40,000	13,000	0·651	19,000	29,000

		Anteroventral and anteromedial		
		Volume	Count	Cells/mm³.
Man	R	68·30	647,000	9000
	L	85·04	680,000	8000

lateral mamillary nucleus (Guillery, 1955), where the cell density is much higher in the cat than it is in the rabbit.

Consideration of cell density in relation to brain weight shows that there is an inverse relationship between brain weight and mean cell density. In the anteroventral nucleus, for example, there are about 100,000 cells per cubic mm. in the rat, about 50,000 in the rabbit, just over 20,000 in the cat, 14,000 in the monkey and about 9000 in man. Two exceptions to this generalization are found in the anterodorsal and medial mamillary nuclei of the cat, in both of which the cell density is significantly lower than in the corresponding nuclei of the monkey.

DISCUSSION

Simpson (1952), Daitz (1953) and Guillery (1955) have previously published counts of the fornix system and, in general, the present more extended results agree well with the earlier values. Simpson found 110,000 fibres in the post-commissural fornix of the macaque and Daitz found 912,000 fibres in the human post-commissural fornix. Since Daitz counted the fibres in the pre-mamillary part of the fornix the figures that have been given here for the human fornix may represent a low value. The cell counts also agree closely with those given previously by Guillery (1955).

In the following discussion each component of the fornix-mamillo-thalamic system has been treated separately and the quantitative results have been discussed in particular relation to the organization within this system.

The pre-commissural fornix

Daitz (1953) and Simpson (1952) have counted the fibres in the subcallosal fornix of human and monkey brains. Daitz found 2,700,000 fibres in man and Simpson found 500,000 fibres in the macaque. The corresponding values for the post-commissural fornix are about 1,200,000 and 220,000, showing that approximately half of the sub-callosal fibres must enter the pre-commissural fornix in each species. It may be significant that the proportion of fornix fibres which enters the pre-commissural component remains roughly constant in spite of a five-fold difference between the monkey and man.

The large size of the pre-commissural component in the monkey has been commented upon by both Simpson (1952) and Bucy & Klüver (1955) but, apart from Simpson's finding of slight degeneration in the lateral septal nucleus following hippocampal lesions, virtually nothing is known of this projection system in primates. More is known of the termination of the pre-commissural fibres in the rabbit and the rat. Sprague & Meyer (1950) have described degeneration in the medial and lateral septal nuclei and in the nucleus accumbens of the rabbit and, more recently, Nauta (1956) has found degeneration in all the septal nuclei, the nucleus of the diagonal band, the preoptic region and the bed nucleus of the anterior commissure in the rat.

Unfortunately, it has not been possible to estimate the size of the pre-commissural component in the rat, the rabbit and the cat but qualitatively this component appears to be as large as, or larger than the post-commissural component; however, such an estimate is difficult on the material available. A further factor which is relevant to the size of the pre-commissural fornix is that not all of its fibres are efferent with respect to the hippocampus (Gerebtzoff, 1939; Morin, 1950; Stoll, Ajmone-Marsan & Jasper, 1951; Daitz & Powell, 1954; Cragg & Hamlyn, 1956). The relative number of the caudally directed fibres is unknown, but they probably form only a small proportion of the fornix fibres.

The post-commissural fornix

In the monkey and in man half of the subcallosal fornix fibres enter the post-commissural fornix but only one-quarter to one-sixth of the sub-callosal fibres

reach the mamillary bodies. This large fibre loss between the anterior commissure and the mamillary bodies has also been found in the rat, the rabbit and the cat.

The detailed pattern of this fibre loss varies between species (compare Figs. 1–5) but in all the animals the greatest number of fibres leave the fornix at, or a short distance caudal to, the level of the medial cortico-habenular tract. There are two major sites to which these fibres may project; the anterior thalamus and the periventricular part of the hypothalamus.

A direct hippocampo-thalamic connexion was described by Gudden (1881) and by Ganser (1882) and a number of subsequent workers have described a similar fibre group. However, some have claimed that this is a connexion between the fornix and the stria habenularis (e.g. Kölliker, 1896; Gurdjian, 1925), while others have described it as a direct projection to the anterior thalamus. Vogt (1898), in a brief communication, described a large fornix projection to the anterior thalamus but was of the opinion that these fibres originated in the septal nuclei. Cajal (1911) also described an anterior thalamic projection but in his figure 501 he shows the fibres arising as collaterals from the main fornix bundle. This important thalamic connexion appears to have been completely overlooked until the work of Nauta (1956) and Guillery (1956) on the rat established that these fibres originate in the hippocampus and pass from the anterior third of the post-commissural fornix to the anteroventral and anteromedial thalamic nuclei. The projection is in part bilateral by way of the inter-anteromedial commissure and some of the fibres are probably related to the mid-line thalamic nuclei.

In the light of these findings it is almost certain that in the rat the majority of the fibres that leave the rostral parts of the post-commissural fornix pass to the anterior thalamic nuclei but two other smaller fibre groups also leave this part of the fornix. The first consists of fibres that arise in the septum and pass to the thalamus and the second consists of fibres that pass from the fornix to the hypothalamus.

Regarding the first of these it is now clear that there are two fibre groups in the region of the 'medial cortico-habenular tract', the medial cortico-thalamic group described above and a septo-thalamic group. It has been shown (Powell & Cowan, 1955) that in the rat, even after bilateral destruction of the fimbria and fornix, a small group of fibres persists in the anterior column of the fornix. Since this group could not be traced beyond the crossing with the stria habenularis it was suggested that these fibres had their origin in the septum and either joined the stria habenularis or terminated in the region of the parataenial nucleus or subjacent hypothalamus. Nauta (1956) has described such a septo-habenular projection but he could not exclude the additional septal projection to the anterior thalamus described by Vogt (1898).

The fibres that pass from the fornix to the hypothalamus fall into a number of groups. The medial cortico-hypothalamic tract forms a well defined bundle in the rat and has been well described in normal material (Cajal, 1911; Gurdjian, 1927; Krieg, 1932). It has been shown that this bundle receives its fibres from the fimbria and ends in the region of the supra-chiasmatic nucleus and in the periventricular region of the hypothalamus (Powell & Cowan, 1955; Nauta, 1956; Guillery, 1956). Other fibres pass from the dorsal aspect of the fornix into the anterior part of the dorsal hypothalamus (Nauta, 1956; Guillery, 1956). The fibre

loss that occurs in the posterior part of the post-commissural fornix may in part be attributed to the termination of fibres in the perifornical nucleus (Sprague & Meyer, 1950).

The post-commissural fornix of the rat loses between 35,000 and 40,000 fibres in its course through the hypothalamus, and it is probable that most of these form a direct hippocampo-thalamic projection system, while the remainder are either hippocampo-hypothalamic or septo-thalamic fibres.

The post-commissural fornix of the rabbit, the cat and the monkey loses between 120,000 and 160,000 fibres, that of man loses about 500,000 fibres. Although there is no evidence for a direct hippocampo-thalamic projection in these species the pattern of the fibre loss suggests that the distribution of the fornix fibres is similar to that found in the rat. Interspecies differences that have been found in the precise pattern of the fibre loss (Figs. 1–5) require further study. The more gradual fibre loss that occurs in the human fornix is probably related to the more oblique path of the fornix through the hypothalamus but this, and other interspecies differences, may also point to a difference in the terminal distribution of the fibres in question.

The majority of the fornix fibres which reach the mamillary region terminate among the mamillary cells but a small proportion by-pass the mamillary nuclei to be distributed to the mid-brain tegmentum and pons (Sprague & Meyer, 1950; Guillery, 1956). The only estimate of the size of the latter component was given by Guillery (1955) who showed that in the rabbit, in which this part of the fornix is particularly large, between 7000 and 11,000 fornix fibres enter the mamillary peduncle, that is, approximately one-tenth of the number of pre-mamillary fibres. Although little is known regarding the termination of these fibres it has been shown that in the rabbit and the rat they travel in the dorsal fornix (Sprague & Meyer, 1950; Powell & Cowan, 1955).

In the rat the majority of the fibres which end in the mamillary nuclei also travel in the dorsal fornix (Powell & Cowan, 1955; Guillery, 1956). They end in relation to all the elements of the mamillary bodies (Nauta, 1956), but the major termination is in the lateral and posterior parts of the ipsilateral medial mamillary nucleus in the rabbit as well as the rat (Sprague & Meyer, 1950; Guillery, 1956). This degree of spatial organization does not appear to be present in the monkey, where Simpson (1952) has demonstrated a diffuse termination of fornix fibres throughout the ipsilateral medial and lateral mamillary nuclei.

The mamillary nuclei

The only previous quantitative studies of the mamillary nuclei are those of Rose (1939–40) and Guillery (1955). Since the present investigation has been strictly confined to the medial mamillary nucleus, whereas that of Rose dealt with the volumes of the mamillary bodies as a whole, the two series are not directly comparable.

The cell counts of the mamillary nuclei can only be discussed in relation to current evidence regarding the organization of their afferent and efferent connexions, for on the basis of these connexions it is possible to describe a number of subdivisions within the medial mamillary nuclei. The experiments of Gudden (1881) and van Valkenburg (1912) first suggested that the two efferent pathways of the mamillary bodies, the mamillo-thalamic and mamillo-tegmental tracts, arise independently

from the posteroventral and anterodorsal parts of the medial mamillary nucleus respectively. More recently it has been shown that in the rabbit and the rat the organization of the mamillo-thalamic projection is such that each of the anterior thalamic nuclei receives its afferents from a distinct subdivision of the larger posteroventral part of the medial mamillary nucleus (Powell & Cowan, 1954; Cowan & Powell, 1954) and further, that the ending of the afferent tracts, the mamillary peduncle and the fornix, is closely related to these subdivisions of the medial mamillary nucleus (Guillery, 1956). Thus, it is now clear that in the rat the fornix fibres end predominantly in the pars lateralis and pars posterior of the medial mamillary nucleus and that these parts project to the anterodorsal and anteroventral thalamic nuclei respectively. The part of the medial mamillary nucleus which projects to the anteromedial thalamic nucleus and to the mamillo-tegmental tract receives its afferents from the mamillary peduncle.

While it would be of interest to know the proportion of the mamillary cells that forms each of these subdivisions, and to relate such proportions to the anterior thalamic counts in particular, the necessary counts have not been practicable for technical reasons. In one rabbit an attempt was made to determine the number of cells projecting to the mamillo-tegmental tract by counting the cells which remain after complete destruction of the anterior thalamic nuclei. However, on account of the resulting shrinkage and gliosis it was felt that estimates of this nature were unreliable.

Although the total medial mamillary counts of the rabbit, the cat and the monkey are similar it is possible that greater interspecies differences would appear if the counts could be split up according to the subdivisions given above, particularly since the subdivisions of the medial mamillary nucleus remain to be studied in the cat and the monkey.

In the rabbit there are more medial mamillary cells than in the cat and the monkey. Guillery (1955) showed that in the rabbit a considerable number of the medial mamillary cells do not send their axons into the principal mamillary tract. He concluded that these cells must be Golgi type II cells or cells which send their axons into the medial forebrain bundle. At present it is not known whether such cells occur in other species, but comparison of the medial mamillary and mamillo-thalamic counts of the rabbit and the rat suggests that there may be similar cells in the rat.

The mamillo-thalamic tract, the mamillo-tegmental tract and the anterior thalamic nuclei

From the present results it is not possible to give any values for the number of fibres in the mamillo-tegmental tract or for the proportion of the medial mamillary cells that send their axons into this tract. From an examination of normal material it is clear that the mamillo-tegmental tract is relatively larger in the rabbit and the rat than it is in the cat and that the tract is particularly small in primates.

In man there are more mamillo-thalamic fibres than there are medial mamillary cells so that the individual mamillo-thalamic fibres must branch on their way to the anterior thalamus. In the rabbit there is probably a similar branching, since the number of fibres in the principal mamillary tract is approximately the same as the number in the mamillo-thalamic tract (Guillery, 1955), and van Valkenburg's

experiment (1912) suggests that at least some of the mamillo-tegmental fibres have an independent origin in the medial mamillary nucleus. There is no direct evidence for branching in the mamillo-thalamic fibres of the rat, the cat and the monkey but the present counts do not exclude such branching and it may well occur in all the species.

At present it is difficult to give an accurate estimate of the ratio between the number of the medial mamillary cells that project to the anterior thalamus and the number of anterior thalamic cells. It is clear that this ratio is close to 1:2 in all the species; it may be slightly less in the rat and rabbit and slightly more in monkey and in man.

It is of interest in this connexion to compare the number of fibres which reach the anterior thalamic nuclei directly from the fornix with the number which reach it from the mamillo-thalamic tract. In all the species, except man, the number of fornix fibres leaving the fornix opposite the rostral thalamus is somewhat greater than the number in the mamillo-thalamic tract. If allowance is made for the fibres of the medial cortico-hypothalamic tract and for the fibres that pass to the habenula or anterior thalamus from the septum, these two figures are approximately equal. Such a comparison shows that the direct thalamic fibres of the fornix not only play a relatively important role in the activity of the anterior thalamus but also suggests that the double projection to the anterior thalamus may be regarded as a constant feature of the mammalian brain.

The anterior thalamic counts have been subdivided into separate figures for each of the anterior thalamic nuclei. These nuclei not only have a discrete projection to the separate areas of the medial cortex (Rose & Woolsey, 1948) but in the rat they each receive a characteristic pattern of afferent impulses from the subdivisions of the medial mamillary nucleus and from the direct thalamic fibres of the fornix.

The anteroventral nucleus receives its impulses from the hippocampus only. Some of the impulses pass through the fornix and the pars posterior of the medial mamillary nucleus and others come directly from the post-commissural fornix. The antero-dorsal nucleus differs in that impulses from the hippocampus reach the nucleus only after being relayed in the pars lateralis of the medial mamillary nucleus; as the latter may also receive some fibres from the mamillary peduncle there is the possibility of some modification by mid-brain activity. The anteromedial nucleus is more closely linked with the mid-brain for although this nucleus receives impulses directly from the fornix it can also be considerably affected by the mid-brain through the mamillary peduncle and the pars medialis of the medial mamillary nucleus.

At present it is not possible to draw up a similar scheme for the anterior thalamic connexions of the other species and the differences in the relative sizes of the anterior thalamic nuclei cannot yet be interpreted in terms of hippocampal and mes-encephalic connexions. However, it is known that the fornix in the monkey ends in relation to the whole of the medial mamillary nucleus and that the mamillary peduncle is particularly small in primates. The anteromedial nucleus is relatively larger in the monkey than it is in the rat, so that the mesencephalic connexions of this nucleus must play a relatively less important part in the monkey than they do in the rat. In primates the activity of the anteromedial thalamic nucleus and thus

of the anterior cingulate cortex may well be under more direct hippocampal influences than in the rat.

The volumes of nuclei and cell density

One of the striking results of this quantitative study has been that while the volumes of the medial mamillary and anterior thalamic nuclei increase with increase in brain weight, the cell density decreases as the brain weight increases. Associated with this is the similarity of the cell counts in the rabbit, the cat and the monkey despite marked differences in brain weight.

With a given increase in brain weight all the nuclei do not show the same percentage decrease in cell density. A comparison of the rabbit and the cat shows that while the medial mamillary and anteroventral nuclei undergo almost the same percentage decrease in cell density, the anteromedial nucleus shows much less and the anterodorsal nucleus considerably more change.

A relationship between brain size and cell density is also found in the cerebral cortex. Thus Bok & Taalman Kip (1940) found that cortical cell density increases in the series mouse, rat, guinea-pig, rabbit, and Tower (1954) found a similar relationship. He claimed that the logarithm of brain weight is directly proportional to the logarithm of cell density and that this relationship holds for brains from the smallest to the largest mammalian species. The present results do not fall close to a straight line when plotted on a double logarithmic scale but they show the general inverse relationship between cell density and brain size.

The decrease in cell density that occurs with increase in brain weight could be due to an increased perikaryal size, to an increase in the axonal or dendritic plexus or to an increase in glia and blood vessels. It is known that perikaryal size increases with increasing brain weight (see, for example, Shariff, 1953), but this is not sufficient to account for the total change in cell density. Haddara (1956) has shown that, per cell, the cortical dendrites of the mouse are fewer and shorter than those of the cat (Sholl, 1953). Differences in the axonal plexus and the complexity of terminal branching may also play a part in determining cell density. The fornix/mamillary ratio, which changes from 1:2 in the rat to 2:1 in man suggests that this may be an additional factor but does not provide conclusive evidence.

It is clear that with an increase in brain size there is an increase in the surface available for interaction between afferent fibres and cells even when the total number of cells in a given system remains constant. Although the function of the system must depend upon its connexions with the rest of the central nervous system the potential complexity of the system appears in general to increase with increasing brain size.

The fornix mamillo-thalamic system as a whole

Overall comparison of the counts draws attention to the relatively large size of the human fornix mamillo-thalamic system. While the small size of this system in the rat suggests that the size of the system may be related to overall brain size or to cortical size, the similarity of the counts for the rabbit, cat and monkey shows that no such simple relationship holds. From the large size of the human fornix mamillo-thalamic system and from the low cell density in its nuclei there can be little doubt

that this system plays a relatively important, though unknown part in the organization of the human brain.

A detailed analysis of the counts within any one species is difficult for two reasons: the difficulty of counting the cells in the smaller subdivisions of the medial mamillary nucleus and the present lack of knowledge regarding the precise organization of the fornix-mamillo-thalamic system in the cat, monkey and man. The gross ratios within the system show reasonable constancy throughout the five species except for the pre-mamillary fornix/mamillary cell ratios. The differences found in this suggest that either the organization of this part of the system changes between the species or that the precise ratio between the mamillary cells and their afferent fibres is not an important factor in determining the activity of the mamillary region.

The present study has been entirely confined to axons and perikarya. Differences in the dendritic pattern of different nuclear groups or of different species have not been considered. However, even a cursory examination of Golgi material shows that there are such differences, and strongly suggests that these may play an important part in the organization of the system as a whole. The complex branching of the anterior thalamic nuclear dendrites compared with the much simpler branching of the mamillary dendrites illustrates this point.

SUMMARY

1. The fibres in the post-commissural fornix and mamillo-thalamic tract and the cells in the medial mamillary and anterior thalamic nuclei have been counted in the rat, rabbit, cat, monkey and man.

2. The absolute figures for the various counts (anterior thalamic nuclei taken together) in the rabbit, cat and monkey are all of the same order; those for the rat are between two and five times smaller and those for man about five times as great.

3. The post-commissural fornix contains about 1,200,000 fibres in man, 200,000–250,000 in the rabbit, cat and monkey and 50,000–60,000 in the rat.

4. In primates the number of fibres in the pre-commissural fornix is approximately equal to the number in the post-commissural fornix.

5. Of the post-commissural fibres between two-thirds and one-half fail to reach the mamillary bodies in all the species. The majority of these are lost in the rostral third of the hypothalamus and probably form a direct hippocampo-thalamic path.

6. The ratio between the number of hippocampo-mamillary fibres and the number of medial mamillary cells varies from 1:2 in the rat to 1:1 in the rabbit, cat and monkey to 2:1 in man.

7. In all the species the ratio between the medial mamillary cells that project to the anterior thalamus and the anterior thalamic cells is approximately 1:2.

8. The anterior thalamic nuclei appear to receive the same number of afferent fibres direct from the fornix as by way of the mamillary nucleus.

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THE VESTIBULAR NUCLEI IN THE CAT

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INTRODUCTION

Some confusion exists in the literature with regard to the delimitation and nomenclature of the vestibular nuclei, making comparisons between results of studies by different workers difficult. A precise chart of the normal topography of the nuclei is essential for an unequivocal presentation of experimental findings. When undertaking studies on the connexions of the vestibular nuclei, we deemed it necessary, therefore, to perform as a first step a thorough mapping of the vestibular complex in the experimental animal employed.

While the basis for any subdivision of a nuclear complex will always have to be the cytoarchitectonic features of its component groups, experimental data on fibre connexions may give additional information. The results of our studies of the connexions of the vestibular nuclei, published separately (Brodal & Pompeiano, 1957; Brodal & Torvik, 1957; Pompeiano & Brodal, 1957*a*, *b*) have, therefore, been taken into account in our analysis. For practical reasons the relevant literature will be dealt with in the Discussion.

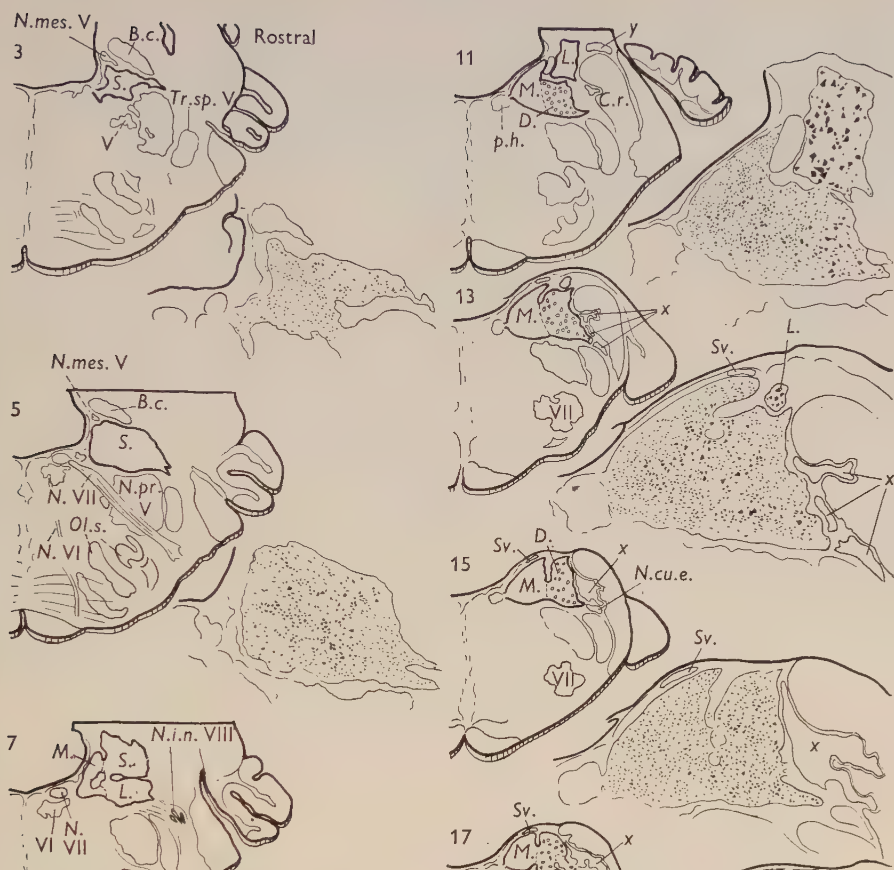
MATERIAL AND METHODS

The animals used in some of our experimental studies (Brodal & Pompeiano, 1957; Pompeiano & Brodal, 1957*a*) were young kittens 2–3 weeks old. The cytoarchitecture of the vestibular nuclei was, therefore, studied chiefly in such animals. Since, however, the chief difference between these young animals and adult ones is a denser packing of the cells, the map applies to adult animals as well.

The maps shown in Text-figs. 1 and 2 were made from serial Nissl-stained transverse sections through the brain stem of a normal kitten $2\frac{1}{2}$ weeks old. The brain was fixed in 96 % alcohol, embedded in paraffin and cut serially at 15 μ. Every fifth section was mounted and stained with Thionine. Drawings were made of sections at equal intervals by means of a projection apparatus, under low magnification. The more detailed drawings of the vestibular complex shown in Text-fig. 1 were made in the same way using a higher magnification, which permitted the marking of individual cells. The drawings were subsequently carefully controlled under the microscope. Photomicrographs were taken from different nuclear groups.

The maps shown in Text-figs. 1 and 2 are true reproductions of the topography of the vestibular nuclear complex in one particular animal. A study of several other, similarly treated, brains from other normal animals as well as from experimental

* This work was done while Dr Pompeiano, Physiological Institute, University of Bologna, Italy, was staying in the Anatomical Institute, University of Oslo, with a grant from the Rockefeller Foundation.



Text-fig. 1. A map showing the topography and cytoarchitecture of the vestibular nuclei in the cat as seen in transverse sections. The numbers of the sections reproduced correspond to those employed in Text-fig. 2. The rings in the descending nucleus represent the fibre bundles of the spinal (descending) root of the vestibular nerve. For particulars see text.

animals makes clear, however, that the series selected may be considered representative. There are only minor variations between different animals.

In addition to Nissl-stained sections series of fibre stained preparations were studied. One of these series was stained according to the Weil method, another according to the Bodian method. Several brains treated according to the method of Glees (1946) were also examined. From one of these the map of Text-fig. 3, showing the nuclei in horizontal sections, was prepared.

Cytoarchitecture and topography of the vestibular nuclei

The vestibular complex is here subdivided, according to common usage, into four principal nuclei, the superior nucleus of Bechterew, the medial (dorsal or triangular) nucleus of Schwalbe, the lateral nucleus of Deiters, and the inferior (or spinal) nucleus or nucleus of the descending root of the vestibular nerve, here referred to as the descending nucleus. In addition some minor groups may be distinguished.

The superior vestibular nucleus is easily outlined, except most rostrally. It is composed of rather loosely scattered, chiefly medium-sized and small cells (Pl. 1, fig. 4). In Nissl-stained sections the medium-sized cells are multipolar or more frequently round or spindle- or pear-shaped with rather fine Nissl granules. The nucleus in young animals sometimes has a somewhat excentric position. The smallest cells appear rounded, stellate, or spindle shaped. In the central portion of the nucleus there are some clusters of somewhat larger multipolar cells. Like the other

Abbreviations used in Text-figs. 1–3

B.c.	Brachium conjunctivum
C.r.	Corpus restiforme
D.	Descending (spinal) vestibular nucleus
f	Cell group *f* in descending vestibular nucleus
g	Group rich in glia cells, caudal to the caudal end of the medial vestibular nucleus
i.c.	Nucleus intercalatus of Staderini
L.	Lateral vestibular nucleus of Deiters
l.	Small-celled lateral group of lateral nucleus
M.	Medial (triangular or dorsal) vestibular nucleus
N.cu.e.	Nucleus cuneatus externus
N.f.c.	Nucleus funiculi cuneati
N.i.n. VIII	Nucleus interstitialis nervi vestibuli
N.mes. V	Nucleus mesencephalicus *n.* V
N.pr. V	Nucleus sensibilis principalis *n.* V
N.tr.s.	Nucleus tractus solitarii
N.tr.sp. V	Nucleus tractus spinalis nervi V
N. VI, VII, VIII	Cranial nerves VI, VII and VIII
Ol.i.	Oliva inferior
Ol.s.	Oliva superior
p.h.	Nucleus praepositus hypoglossi
S.	Superior vestibular nucleus of Bechterew
Sv.	Cell group probably representing the nucleus supra-vestibularis
Tr.s.	Tractus solitarius
Tr.sp.n. V	Tractus spinalis *n.* V
V, VI, VII, XII	Cranial motor nerve nuclei
X	Dorsal motor (parasympathetic) vagus nucleus
x	Small-celled group *x*, lateral to the descending vestibular nucleus
y	Small-celled group, lateral to the nucleus of Deiters
z	Cell group dorsal to the caudal part of the descending vestibular nucleus

cells the largest elements in transverse sections tend to be arranged in elongated groups from dorsomedial to ventrolateral (see drawing 5 in Text-fig. 1), due to the presence of fibre bundles with this course.

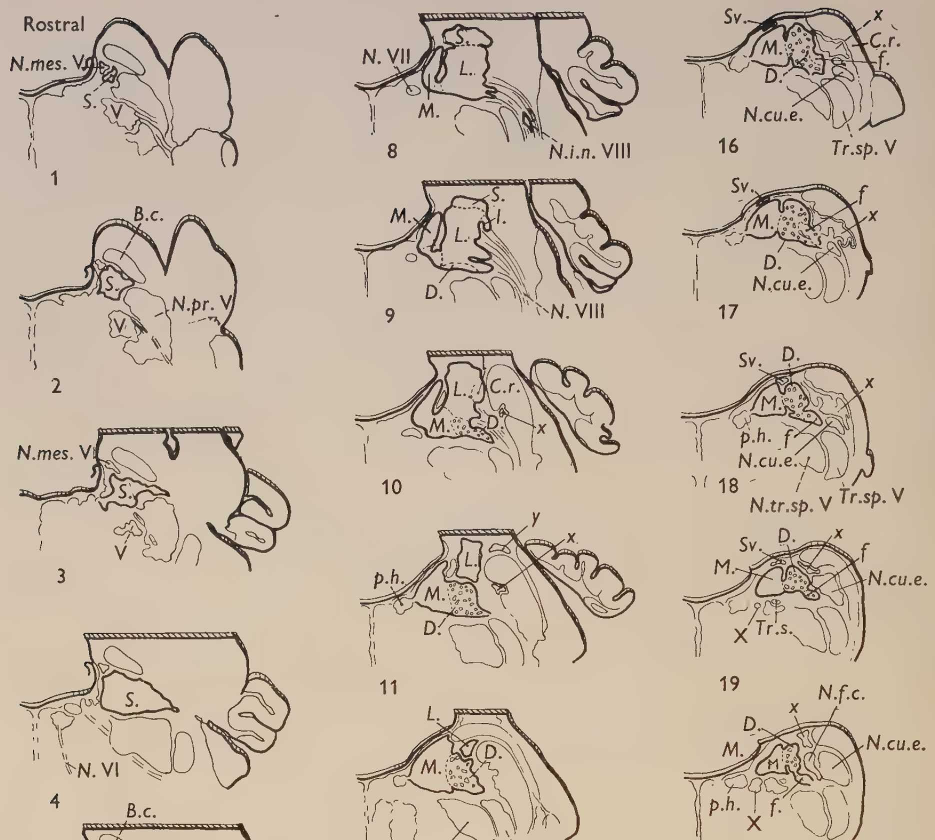
The superior nucleus extends from a level a little rostral to the caudal pole of the motor trigeminal nucleus (drawings 1–3 in Text-figs. 1, 2) to a level slightly caudal to the nucleus of the abducent nerve (drawing 9). In its middle part the nucleus has a triangular shape.

Along its entire rostro-caudal extent the superior vestibular nucleus is capped dorsally by the superior cerebellar peduncle. Medially the nucleus comes close to the floor of the fourth ventricle, except most caudally where the medial nucleus is interposed between it and the ventricle (drawings, 6, 7 in Text-figs. 1, 2). Here the two nuclei fuse. Dorsomedial to the rostral part of the superior nucleus is the mesencephalic trigeminal nucleus (drawings 1–5 in Text-figs. 1, 2). Ventrally to the caudal half of the superior nucleus is the lateral nucleus of Deiters. Their different cytoarchitecture makes the border between the two nuclei fairly distinct (most caudally they are separated by fibre bundles) except rostrally, because there are relatively few giant cells in the rostralmost part of the lateral nucleus. The rostral half of the superior nucleus is indistinctly separated from the principal trigeminal nucleus, which comes into contact with its ventral border. The lateral border of the superior nucleus is well defined by the presence of the fibre masses of the restiform body.

In fibre preparations the fibre bundles referred to above, coursing in a direction from ventro-lateral to dorso-medial, are distinct, and many of these fibres are myelinated. Otherwise the fibre texture of the superior nucleus shows no particular pattern.

The lateral vestibular nucleus of Deiters. This term is here taken to denote that part of the vestibular nuclear complex which is characterized by containing the giant cells of Deiters. These are multipolar, have coarse Nissl-granules, arranged concentrically around the nucleus, which is most commonly fairly centrally placed (Pl. 1, figs. 1, 2). They show considerable variations in size, having in 2–3 weeks old kittens a largest diameter across their perikaryon varying between some 30 to about 45 μ. The lateral nucleus also contains smaller cells of varying types. Some are fairly large, usually multipolar, others are medium-sized, frequently oval or spindle shaped, while still others are very small (Pl. 1, fig. 1). The giant cells are relatively more numerous and in general somewhat larger in the caudal part of the nucleus than in its rostral part (Pl. 1, figs. 1, 2, and drawings 7–11 in Text-fig. 1). The number of smaller cells, however, is definitely less in the former part. The two regions of the nucleus fuse imperceptibly with each other. At the lateral border of the nucleus a small group consisting of medium-sized cells only can easily be distinguished in most animals (Pl. 1, fig. 3, and drawings 9–10 in Text-figs. 1, 2). This group is here labelled *l*.

The lateral vestibular nucleus begins rostrally at the level of the middle of the superior nucleus (drawing 6 in Text-fig. 2). Its caudal end is found caudal to the nucleus of the abducent nerve (drawing 13 in Text-figs. 1, 2). Fibres of the striae medullares may separate the caudalmost part of the nucleus into two, a dorsal group, apparently situated in the base of the cerebellum, and a ventral group,



Text-fig. 2. A series of drawings, taken with equal intervals, of transverse Thionine stained sections through the vestibular complex of the cat. The outlines of the vestibular nuclei are shown as heavy lines. Abbreviations as in Text-fig. 1.

situated in the angle between the medial and descending nucleus and the restiform body.

The border between the lateral nucleus and the superior nucleus, dorsal to it, was considered above. The lateral nucleus extends further caudally than the superior. Its caudalmost part, therefore, borders dorsally on the white matter of the cerebellum ventral to the nucleus interpositus. Ventro-laterally the rostral part of the lateral nucleus approaches the trigeminal principal sensory nucleus and the spinal tract. Ventral to the caudal third of the lateral nucleus the descending nucleus is interposed between it and the spinal trigeminal nucleus (drawings 9–11 in Text-figs. 1, 2). The fibres of the facial nerve are found ventro-medial to the rostralmost part of the lateral nucleus. Caudally it borders medially on the medial vestibular nucleus. It can clearly be distinguished from this on account of its content of giant cells, and more dorsally by a narrow zone of fibres, almost free from nerve cells. Lateral to the lateral nucleus is the restiform body.

In fibre preparations the vestibular root fibres, which enter the lateral nucleus from its lateral aspect (drawings 6–10 in Text-figs. 1, 2) are seen to radiate fanlike within the territory of the nucleus, frequently producing a separation of its cells into minor aggregations. Other fairly heavy bundles of myelinated fibres enter the lateral nucleus from its dorsal aspect. Most of these are certainly cerebello-vestibular fibres.

The medial vestibular nucleus is clearly recognized as a particular entity in its medial part. At caudal levels its lateral borders are less clear. It is made up of cells of different sizes (Pl. 1, figs. 5, 6), the majority being medium-sized, triangular, multiform or more rounded, with a fairly centrally placed nucleus and rather fine Nissl granules. The smaller cells in the nucleus usually are round or pear-shaped in Nissl-stained sections, and their cytoplasm is scanty. The cells of the medial nucleus lie rather close together. At its middle levels, particularly dorso-laterally towards the descending nucleus, there are some more of the largest elements (Pl. 1, fig. 6) than further rostrally and caudally (Pl. 1, fig. 5). In many animals a tiny group of larger cells may be seen in the medialmost corner of the nucleus dorsal to the dorsal motor vagal nucleus (drawing 19 in Text-fig. 1).

The rostral end of the medial vestibular nucleus is found approximately at the same level as the rostral pole of the lateral nucleus (drawing 6 in Text-fig. 2) and is situated just underneath the floor of the fourth ventricle. Throughout its extent to its caudal extremity, at the level of the rostral pole of the hypoglossal nucleus, the nucleus retains its characteristic relation to the ventricle. The caudal end of the nucleus (see also Text-fig. 3) is found a little rostral to the caudal disappearance of the descending nucleus.*

Near its rostral end the medial nucleus is not clearly separated dorso-laterally from the superior nucleus (drawings 6 and 7 in Text-figs. 1 and 2). Ventrally it is continuous with the reticular formation, and medially it is connected by cell

* Below the caudal end of the medial nucleus is a dense accumulation of glia cells with a few nerve cells (*g* in Text-figs. 1–3), which continues caudally between the nucleus of the solitary tract, the descending vestibular nucleus and our group *z*, to be described below. Medial to the group *g* there is a slender column of relatively big multipolar cells. These cells may perhaps represent a caudal prolongation of the medial vestibular nucleus, which, as noted above, frequently contains some larger cells medially in its caudalmost part.

strands with the nucleus praepositus hypoglossi (drawings 11–18 in Text-figs. 1, 2). Ventral to the caudal extremity of the medial nucleus is the solitary tract and its nucleus and the dorsal motor vagal nucleus (drawings 19–20 in Text-figs. 1, 2). The lateral border of the medial vestibular nucleus is easily recognized at rostral levels, where a fibre bundle separates it from the lateral vestibular nucleus. More caudally the ventral part of the nucleus fuses laterally with the descending nucleus (drawings 9–20 in Text-figs. 1, 2) while its dorsal part is separated by a narrow cell-free zone from the latter. This zone may occasionally be identified even to the caudal end of the medial nucleus.

In myelin-sheath stained preparations finer fibres are seen coursing in all directions within the medial nucleus. More compact strands of fibres are seen only in its lateral-most region. These fibres run in a ventral direction to the ventro-lateral angle of the nucleus, where they turn medially and course closely ventral to the nucleus and to the nucleus praepositus hypoglossi. They appear to enter the area of the medial longitudinal fasciculus. The border between the medial and the descending nucleus is clearly seen in fibre preparations, particularly with myelin-sheath staining (Pl. 1, fig. 9).

The descending (*spinal*) *vestibular nucleus* is the most difficult part of the vestibular complex to outline. As defined by us (Text-figs. 1, 2) it is a rather large nuclear group.

Its cytoarchitecture is not quite uniform throughout. In addition to small and medium-sized cells, resembling those of the medial nucleus, the descending nucleus contains a certain number of larger cells (Pl. 1, fig. 7, and drawings 11–15 in Text-fig. 1). Some of these are multipolar and almost approach the giant cells of the lateral nucleus in size.* Such large cells occur throughout the nucleus, but are particularly abundant in its rostral part (drawings 11–13 in Text-fig. 1). Ventro-laterally in its caudalmost part (drawings 17–21 in Text-fig. 1) numerous fairly large cells are so densely packed (Pl. 1, fig. 8) that it appears justified to consider this region as a particular subdivision. Since it appears to be the same group which is labelled *f* by Meessen & Olszewski (1949, their Pl. VII) in the rabbit we have employed the same designation. The group *f* ventro-laterally approaches the spinal trigeminal tract and its nucleus (drawings 17–21 in Text-fig. 1). It shows some variations in shape between different animals and frequently forms a longitudinal column which may, however, be interrupted. Apart from the region of the group *f* the descending nucleus is characterized by the scattering of its cells due to the presence within it of large numbers of longitudinally running fibres (shown as rings in the drawings in Text-figs. 1, 2).

The descending nucleus begins rostrally at the level where the caudal vestibular root fibres enter the vestibular complex (drawing 9 in Text-fig. 2) as a transversely extended zone of cells immediately ventral to the lateral nucleus. A little further caudally (drawing 11 in Text-figs. 1, 2) bundles of longitudinally running fibres, presumably belonging to the spinal vestibular root, intrude between the two nuclei. The delimitation of the nucleus medially, where it more or less fuses with the

* Occasionally a characteristic giant cell of the type present in the lateral nucleus may be seen in the rostral part of the descending nucleus. These cells are probably to be considered as displaced Deiters' cells.

medial nucleus, was described above. At caudal levels the cytoarchitectonic differences between these two nuclei are less distinct than more rostrally (see drawings 11–19 in Text-fig. 1). Lateral to the descending nucleus is rostrally the restiform body, more caudally the group labelled x in our map, the external cuneate and the cuneate nucleus. At some places the nucleus almost fuses with the group x. Its caudal extremity lies immediately ventral to the group here labelled z (drawings 21–23 in Text-figs. 1, 2). On its medial side is the group of glia cells referred to above. It should be noticed that the descending nucleus dorsally extends to the surface of the rhombencephalon along its rostrocaudal extent, except for its caudal extremity and its rostralmost part.

In fibre preparations the descending nucleus is characterized by numerous longitudinally running fibre bundles (Pl. 1, fig. 9).

Some smaller cellular groups. Between the root fibres of the vestibular nerve there are some strands of cells, not very numerous, which represent the *noyaux interstitiels du nerf vestibulaire* of Cajal (1909). These cells (drawings 6–8 in Text-figs. 1, 2) are medium-sized and commonly elongated, with their long axis oriented along the vestibular root fibres. In some animals the nucleus contains scattered giant cells like those in the nucleus of Deiters, which may be connected with the interstitial nucleus by a strand of cells. Other strands may form a connexion with the descending nucleus, and scattered cells may extend towards the superior nucleus.

Dorsal to the vestibular nuclei there are some rather loosely structured *strands of cells which extend into the white matter of the cerebellum,* where they approach the central cerebellar nuclei and form a connexion between the superior and lateral nucleus with the nucleus fastigii and the ventro-lateral part of the nucleus interpositus.

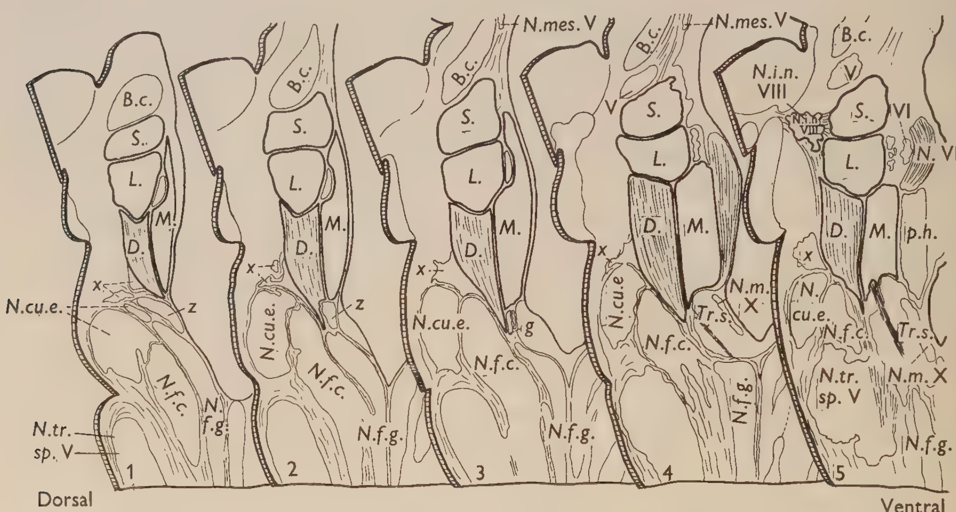
A small *group of cells,* here labelled *Sv*, is found *immediately beneath the dorsal surface of the medulla oblongata* at levels between the caudal end of the nucleus of Deiters and the level of the rostral pole of the external cuneate nucleus (drawings 13–19 in Text-figs. 1, 2). It is separated ventrally from the lateralmost part of the medial vestibular nucleus by some transversely running fibres. It may consist of a rostral and a caudal portion, and has more or less spindle-shaped cells, with their longitudinal axis oriented along the surface of the medulla. We believe this group to correspond to Olszewski & Baxter's (1954) nucleus supravestibularis in man.

A *small-celled group, here called y, is situated dorsocaudal to the restiform body* where this begins to fan out into the cerebellar white matter (Text-figs. 1, 2). In transverse sections it is seen to cap the restiform body dorsally. It is situated immediately lateral to the caudal part of the nucleus of Deiters, and is ventro-laterally practically in contact with the dorsal cochlear nucleus. From the dorsal aspect of this group, scattered cells form strands extending to the ventralmost part of the dentate nucleus.

Between the caudal half of the descending vestibular nucleus and the rostral pole of the external cuneate nucleus is a zone of cells, here called x, which differs from these nuclei with regard to its cytoarchitecture (Text-figs. 1–3). It contains scattered small cells of somewhat varying shape. It can best be distinguished from the adjoining nuclei in horizontal silver impregnated sections (see Text-fig. 3). The zone begins caudally a little below the rostral pole of the external cuneate nucleus and

increases in size more rostrally, its upper, broadest part covering the top of the external cuneate nucleus. Here it has the restiform body on its lateral side.

The small cell group, here labelled *z*, is found *immediately rostral to the rostral end of the nucleus gracilis* (drawings 21–23 in Text-figs. 1, 2, see also Text-fig. 3). It is situated closely underneath the dorsal surface of the medulla oblongata, dorsal to the caudal end of the descending vestibular nucleus and the group *g*. In Nissl-sections its cells are medium-sized, ovoid to polygonal, and on the whole rather pale. Caudally this group is usually separated from the rostral end of the nucleus gracilis by a narrow cell-free interval. Its cytology resembles somewhat that of the latter nucleus, a fact which makes it difficult to keep the two apart in Nissl-stained sections. However, in horizontal silver-stained sections (see drawings 1–2 in Text-fig. 3) the group *z* clearly differs from the gracilis nucleus in its fibre pattern. It is also unlike the group *x* (cf. above).



Text-fig. 3. A series of drawings, taken with equal intervals, of horizontal silver impregnated sections through the vestibular complex of an adult cat. This diagram is included especially in order to make clear the topographical relations of the small cell groups *x* and *z*, which may be mistaken to be parts of the descending vestibular nucleus. Abbreviations as in Text-fig. 1.

DISCUSSION

The subdivision of the vestibular nuclear complex shown in our maps (Text-figs. 1, 2) in general agrees with those made by several other students of these nuclei in mammals, including man. Since there are, however, conflicting views concerning some points, it may be appropriate to give our reasons for choosing the delimitation employed.

The name *lateral vestibular nucleus of Deiters* has been used very inconsistently. While von Monakow (1883, and later) considered as the nucleus of Deiters the part of the vestibular nuclear complex in which the giant nerve cells undergo chromatolysis following lesions at rostral levels of the cord, other anatomists define the nucleus of Deiters as comprising extensive parts of the entire vestibular complex.

We have employed the term lateral nucleus of Deiters (also called 'grosszelliger Vestibulariskern' or 'noyau à grandes cellules de l'acoustique') for that part of the vestibular complex in which the multipolar giant cells of Deiters form a characteristic cytoarchitectonic element. It should be emphasized, however, that there are at least as many cells of other types within its territory (cf. description and Text-fig. 1). This restricted use of the term is in agreement with the usage of authors such as Sabin (1897), Cajal (1896, 1909) and Kappers, Huber & Crosby (1936).

A few large cells, some of them attaining the size of the giant cells of Deiters, commonly occur in what is here called the descending nucleus, and occasionally in the regions of the medial nucleus bordering on the lateral nucleus. However, the architecture of the lateral nucleus proper (see Text-fig. 1 and Pl. 1, figs. 1, 2) is characteristic enough to justify its distinction as a separate nucleus, and the scattered giant cells found elsewhere are probably to be considered as displaced specimens. Support for this view is derived from the results of experimental studies, since following lesions of the spinal cord at high cervical levels not only the giant cells, but the other cells of the lateral nucleus (as defined here) as well, are affected with retrograde cellular changes (Pompeiano & Brodal, 1957*a*), while no changes occur in other vestibular nuclei. Furthermore, all descending fibres from the lateral nucleus appear to course in the vestibulo-spinal tract. Such findings indicate that the nucleus of Deiters as outlined here forms a particular unit of the entire vestibular complex, even if it, like other vestibular nuclei contributes fibres to the ascending medial longitudinal fasciculus (Brodal & Pompeiano, 1957).

The uniform efferent fibre connexions of the nucleus of Deiters as outlined on a cytoarchitectonic basis are of interest with regard to the question of its subdivision into minor groups. As mentioned above, there are some differences in architecture between various regions of the lateral vestibular nucleus as noticed also by some previous authors, for example Sabin (1897), who in man found differences which appear to be similar to those described here. Lewy (1910) (in the rabbit) comments on the fact that the shape of the giant cells is not the same throughout the nucleus. However, apart from the little group of medium-sized cells, here called *l* (Text-figs. 1, 2, Pl. 1, fig. 3), the other differences are slight, and in view of the uniform efferent fibre connexions may not be significant. The fact that the vestibulo-spinal fibres coursing to different levels of the cord show a somatotopical arrangement with regard to their site of origin within the nucleus (Pompeiano & Brodal, 1957*a*) may have some bearing, not yet capable for explanation, on the regional differences in architecture.

The group *l* probably should be considered a particular part of the lateral nucleus, even if its efferent fibres course in the vestibulo-spinal tract, since at least three-quarters of its cells send fibres to levels of the cord below Th_1 (Pompeiano & Brodal, 1957*a*).

Other data support the delimitation of the nucleus of Deiters as made in this study, for example the observation of Vraa-Jensen (1956) that in the chicken the cells of the nucleus of Deiters are derived from the medial part of the matrix in the basal lamina, and not, like the other vestibular nuclei, from the dorsal part of the alar lamina.

There are thus good reasons for delimiting the nucleus of Deiters as done here,

and to consider this as a particular unit of the vestibular complex. Mention should, however, be made of some papers in which another delimitation is chosen. Fuse (1912) distinguishes seven subgroups of the Deiters' nucleus basing, it appears, this extensive use of the name on the occurrence of large cells. His 'Triangularis-Anteil' obviously is part of what is here considered the medial vestibular nucleus, which at some levels contains large cells (see Text-fig. 1), and his medioventral group is clearly the rostral part of the descending nucleus as outlined here, in which there are a certain number of large cells, and even scattered (presumably displaced) Deiters' cells may occur. The interstitial nucleus of the vestibular nerve is called by Fuse the intravestibular group of the nucleus of Deiters. His other subdivisions: the dorsal, the mediodorsal, the central and the dorso-lateral group appear to be parts of what we have defined as the nucleus of Deiters. Also Lewy (1910) distinguishes different regions of the nucleus of Deiters, his 'ventrale Abteilung' according to his illustrations being the rostral part of the descending nucleus. Muskens (1914) speaks of the medial nucleus as part of the nucleus of Deiters. Winkler & Potter (1914) in their atlas of the cat's brain (their Pl. XXVII) label as ND (Deiters' nucleus) part of what is here considered as the descending nucleus.

In their atlas of the rhombencephalon of the rabbit Meessen & Olszewski (1949) label various groups as parts of the nucleus of Deiters. As judged from their cytoarchitecture and from their position only groups (subnuclei) Dtγ on Pls. IX and X, and Dtα on Pls. X and XI belong to the nucleus of Deiters proper. On Pls. VIII and IX the group labelled Dtα differs clearly cytoarchitectonically from the group carrying the same name on Pls. X–XI. There is little doubt that the group Dtα on Pls. VIII and IX as well as the group Dtβ on Pl. IX belong to the descending nucleus. It is seen from the photographs that, contrary to what is said in the text, the group Dtγ does not consist exclusively of giant cells. The reasons for its separation are not entirely clear.

The *superior vestibular nucleus* (angular nucleus, nucleus of Bechterew) by virtue of its uniform cytoarchitecture and relatively distinct borders is fairly unanimously delimited as done here by most authors (Cajal, 1909, in the mouse; Meessen & Olszewski, 1949, in the rabbit; Winkler & Potter, 1914, in the cat; Sabin, 1897, Jacobsohn, 1909, Marburg, 1910, and Olszewski & Baxter, 1954, in man). In the literature references are found to some cell groups, usually labelled as nuclei, which have been distinguished at levels where the superior nucleus is present. Thus Lewandowsky (1904) in the cat refers to a cell group which he calls '*nucleus supremus acustici*'. His short description and his figure 11, showing the nucleus in a Marchi preparation, do not enable one to get a clear impression of its extension. Like Fuse (1912) we have not been able to convince ourselves of the existence of this nucleus of Lewandowsky. It may be part of what we have outlined as the superior nucleus, but we do not feel that the regional cytoarchitectonic differences within the superior nucleus are sufficiently clear to warrant a subdivision of this nucleus into minor groups, the more so since the ascending fibres from the superior nucleus appear to take origin from all parts of it (Brodal & Pompeiano, 1957). Similar considerations as made above with regard to the 'nucleus supremus acustici' apply to what Fuse calls the 'nucleus of Onufrowicz' (1885) and the 'nucleus of Kohnstamm' (1910). Until further studies may reveal that the superior vestibular

nucleus is not an entity with regard to fibre connexions and intrinsic organization (arrangements of axons and dendrites, etc.), it seems advisable to consider it as one unit of the vestibular nuclear complex.

The *medial vestibular nucleus* (dorsal vestibular nucleus, triangular nucleus, principal vestibular nucleus, nucleus of Schwalbe) as outlined here corresponds to the nucleus as described and pictured for example by Sabin (1897), Cajal (1909), Ferraro *et al.* (1940) and Meessen & Olszewski (1949). Concerning the medial delimitation of the medial nucleus the descriptions in the literature largely conform. Likewise is there general agreement on the fact that the medial nucleus is situated beneath the floor of the fourth ventricle. Concerning the lateral borders of the nucleus there are, however, conflicting views. Reference was made above to the erroneous inclusion of part of the medial nucleus in the concept of the lateral nucleus. Some authors include into the medial nucleus part of what we have here delimited as the descending nucleus, namely its dorsocaudal portion. This is the case, for example, with Winkler & Potter (1914). In their atlas of the cat's brain (their Pl. XXVII) these authors distinguish four-cell groups within the medial nucleus. However, the medial part of their group *a* is the nucleus praepositus hypoglossi (cf. Brodal, 1952), while group *c* and part of *d* (and probably also the region labelled ND, Deiters' nucleus) appear to be cellular areas which belong to the descending nucleus. Thus, only group *b* and the lateral part of group *a* represent the medial vestibular nucleus proper. The ventral border of the medial vestibular nucleus may be difficult to define exactly in Nissl preparations since it fuses with the reticular formation.*

The area identified as the *descending vestibular nucleus* has a cytoarchitecture which, particularly in its rostral part, differs from that of the medial nucleus (cp. Pl. 1, figs. 5, 7), even if there are similarities in Nissl-stained sections, and the border between the two nuclei at some levels is indistinct. However, when the presence of the longitudinally running fibre bundles (shown in Text-fig. 2, see also Pl. 1, fig. 9) is used as a criterion, the descending nucleus can be unequivocally outlined. The delimitation of the descending (spinal) vestibular nucleus as given here largely agrees with that given by Cajal (1909) in the mouse and by Sabin (1897) in man.

It is worthy of notice that as judged by its cytoarchitecture as well as by the presence of descending vestibular root fibres the descending nucleus begins rostrally where the bulk of the vestibular root fibres enter the vestibular complex, namely, ventral to the caudal third of the nucleus of Deiters. Quantitatively it is thus an important part of the vestibular complex.† As mentioned above, some authors have considered the rostral part of the descending nucleus as belonging to what they call the nucleus of Deiters. In some descriptions the dorsal and medial regions of the nucleus as outlined here are taken to belong to the medial nucleus, probably because these parts of it extend dorsally to the surface of the rhombencephalon, and the

* It appears from studies of the distribution of primary vestibular fibres (unpublished) that the ventralmost strip of what in Nissl-preparations appears as the medial nucleus, is devoid of vestibular afferents, and thus should not be included in this nucleus.

† It is remarkable that the spinal afferents to the descending nucleus are limited to its extreme caudal ventro-lateral part only (Pompeiano & Brodal, 1957*b*), while this region of it appears to be poor in primary vestibular afferents (unpublished observations).

cytoarchitecture of the medial and descending nucleus at these levels is rather similar. For example, Winkler & Potter (1914), as mentioned above, include parts of what is here interpreted as the descending nucleus into the medial nucleus, considering apparently only the ventro-lateralmost region (composed of somewhat larger, densely packed cells) as the descending nucleus. Authors erroneously outlining the medial nucleus in this way are then left with a descending nucleus of modest dimensions, which to a large extent is made up of the rather compact cell group *f*, described above (drawings 16–21 in Text-figs. 1, 2, and Pl. 1, fig. 8). It may be discussed whether this part of the vestibular complex should be considered a particular nucleus. Experimental studies (Brodal & Torvik, 1957) show that many of its cells project on to the cerebellum, while others give off fibres ascending in the medial longitudinal fasciculus (Brodal & Pompeiano, 1957). However, cells belonging to the surrounding parts of the descending nucleus behave in the same manner. The group *f*, thus, cannot be separated from the adjoining areas of the descending nucleus by virtue of its efferent connexions, and until future studies may show that it owes its peculiarities to other features in the organization of the vestibular nuclei, it appears reasonable to consider it as a particular group of the descending nucleus only. As mentioned in the description it may frequently be interrupted into minor cell groups. The groups *b* and *c* of Meessen & Olszewski (1949, their pls. V, VI) are probably such minor groups of the larger collection of compactly placed larger cells, called here collectively group *f*.

It is of some importance to be aware of the fact that the medial part of the external cuneate nucleus is frequently broken up into minor clusters of cells which are very close to the lateral border of the descending nucleus. The presence between these two nuclei of the small-celled zone *x* (see Text-figs. 1–3) may facilitate the distinction between the descending vestibular nucleus and the external cuneate nucleus.

The *noyaux interstitiels du nerf vestibulaire* of Cajal (1909) have been observed by several authors (Fuse, 1912; Klossowsky, 1933; and others). In our experimental studies we have observed that some of its cells are affected by retrograde changes following lesions of the medial longitudinal fasciculus (Brodal & Pompeiano, 1957) or of the spinal cord (Pompeiano & Brodal, 1957*a*), while lesions of the cerebellum do not produce changes in the nucleus (Brodal & Torvik, 1957). According to Cajal (1909) primary afferent vestibular fibres establish synaptical contact with its cells. The fact that the interstitial nucleus frequently contains some giant cells of the type found in the lateral nucleus, and the fact that its fibre connexions are similar to those of the latter suggest that it is possibly an aberrant part of Deiters' nucleus. This opinion was held also by Cajal (1909) and Fuse (1912).

The *small-celled zone x* (Text-figs. 1–3), lateral to the caudal part of the descending nucleus, might, on account of its close contact with this, be considered as part of it, and probably has by some authors been interpreted in this way. However, following lesions of the eighth nerve, terminal degeneration is not found in the zone *x* (Glees method, unpublished observations), while the descending nucleus is full of degenerating particles. Nor does the zone belong to the external cuneate nucleus, since lesions of the dorsal funiculi do not produce degeneration in it (Pompeiano & Brodal, 1957*b*) while degeneration is abundant in the external cuneate nucleus. On the other hand, the zone *x* receives spinal afferents ascending in the lateral funiculus of

the cord (presumably collaterals of the dorsal spino-cerebellar tract). These findings on the afferents to this zone make it appear likely that our zone x represents a particular 'nucleus', even if its efferent connexions appear to be similar to those of the adjoining regions of the descending vestibular nucleus, since it sends fibres to the 'vestibular' areas of the cerebellum (Brodal & Torvik, 1957) and to higher levels of the brain stem (Brodal & Pompeiano, 1957). The fact that the external cuneate nucleus projects on to other regions of the cerebellum (Brodal, 1941) than the zone x furnishes additional evidence that this zone is not related to the external cuneate nucleus.

We have not been able to locate specific references to this zone x in the literature. It is interesting to notice, however, that it appears to be present also in the rabbit. From Pl. VII in Meessen & Olszewski's (1949) atlas it is seen that the lateralmost part of the area outlined as the *N.Rd.* VIII (descending nucleus) has smaller and less densely packed cells than the medial part. (Maybe the zone is present also on Pl. VI.) It is possible that the zone x is what has by some authors been referred to as the nucleus (proprius) corporis restiformis. Frequently, cell strands can be followed laterally from this zone into the area of the restiform body, which is also attached to the lateral part of the zone rostrally. Much confusion exists in the literature with regard to the so-called nucleus corporis restiformis (which sometimes has been confused even with the external cuneate nucleus). It is considered to be beyond the scope of the present paper to discuss this subject further.

The *small-celled group y*, lateral to the caudal part of the lateral vestibular nucleus, is difficult to evaluate. It is drawn but not labelled by Winkler & Potter (1914, their Pl. XXV), and it has been noticed by Fuse (1912) who shows it in his figure 27, corresponding precisely to our findings. Fuse considers this cell group to be related to the dentate nucleus, with which, as mentioned above, it is connected. It is not possible to decide whether it may correspond to what Cajal called the nucleus cerebello-acusticus (1896, p. 66). The group Dtθ on Pl. X in Meessen & Olszewski's (1949) atlas, according to its position, may be our group y in the rabbit, but if so, its cytoarchitecture appears to be somewhat different from that in the cat. (Certainly their group Dtθ does not belong to the nucleus of Deiters, however.) Contrary to Fuse (1912) we have not found convincing changes in this group following lesions of the cerebellum (Brodal & Torvik, 1957). Nor have we observed altered cells in it following lesions of the upper brain stem (Brodal & Pompeiano, 1957) or of the spinal cord (Pompeiano & Brodal, 1957*a*). On the whole it appears probable that our group y does not form part of the vestibular complex.

A nucleus *supra-vestibularis* has been described by some authors (for example Olszewski & Baxter, 1954, in man). We are inclined to believe that the group *Sv* (see Text-figs. 1, 2) might correspond to the supra-vestibular nucleus in man, since its topography and cytology appear to be similar. We have not been able to trace primary vestibular fibres to this group.

The small *cell group z*, situated immediately rostral to the rostral end of the nucleus gracilis is difficult to distinguish from the latter in Nissl-stained sections. It was only when studying horizontal silver-impregnated sections (see Text-fig. 3) that we became aware of the individuality of this small group. Like our group x the cell group z appears to be a particular nucleus. We have not been able to trace primary vestibular fibres to it (unpublished observations), and it thus does not appear to

belong to the vestibular nuclear complex. Unlike the adjoining part of the descending nucleus, it does not send fibres to the cerebellum (Brodal & Torvik, 1957). It receives fibres from the spinal cord (Pompeiano & Brodal, 1957*b*). Since these fibres ascend in the lateral funiculus the group *z* should probably not be considered a particular part of the nucleus gracilis.*

The small cell groups discussed here have been dealt with in some details, because if they are not kept apart from the vestibular nuclei proper, erroneous conclusions of experimental anatomical and physiological studies are apt to occur.

General comments. While our subdivision of the vestibular complex is based primarily on studies of the cytoarchitecture of the nuclei, experimental data on afferent and efferent fibre connexions have made it possible to clarify doubts as to the delimitation of particular cell groups. Such information on the whole supports the delimitations arrived at from studies of the cytoarchitecture, for example, with regard to the nucleus of Deiters and our zone *x*. On other points, however, the fibre connexions furnish evidence that a nucleus which has so far been considered a particular unit might deserve a finer subdivision into minor parts, as is the case with the descending and medial vestibular nuclei. Further studies of the fibre connexions of the vestibular complex are needed to clarify the questions. It will be important also to have more detailed information of the type and course of dendrites and collaterals of axons of the cells of the vestibular complex, obtainable only by means of the Golgi method, not least in order to make clear the possibilities of interaction between the various vestibular nuclei and the relations between these and the reticular formation of the brain stem. Even if some data of this kind are known, chiefly thanks to the studies of Cajal (1896, 1909) and Lorente de Nó (1924, 1931, 1933), it is essential that such features as well as the fibre connexions are studied with particular reference to the topographical subdivisions which can be distinguished within the vestibular nuclear complex.

In physiological studies of the vestibular nuclei it is equally important to pay due respect to topographical features in their organization. The disregard of this requirement by many physiologists has certainly hampered progress in our knowledge of the functional organization of the vestibular nuclei. The anatomical data known so far make it appear likely that these nuclei are mutually rather dissimilar also in a functional respect.

SUMMARY

The cytology and topography of the vestibular nuclei have been studied in serial Nissl-stained transverse sections through the brain stem of young cats. The subdivision of the nuclear complex arrived at from cytoarchitectonic studies is considered in the light of experimental studies of the fibre connexions of the vestibular nuclei, which have given important clues as to a rational subdivision. The subdivision employed is shown in Text-figs. 1–3, and in general agrees with that of most previous students.

The term *lateral vestibular nucleus of Deiters* should be restricted to that part of the vestibular complex which harbours the characteristic giant cells. The nucleus

* It is possible that our group *z* is represented by the dorsalmost part of what is labelled '*Gr.+Cu.m*' in Pl. IV in Meessen & Olszewski's (1949) atlas. In the plates of Winkler & Potter's (1914) atlas it is not possible to define a corresponding cell group.

contains also many smaller cells, some of which form a particular group *l*. The cells of this, as well as the other cells of the nucleus, send their fibres to the cord in the vestibulo-spinal tract in a somatotopically arranged pattern.

The *superior vestibular nucleus* is fairly easily outlined. No reasons for subdividing this nucleus further have been found.

The *medial vestibular nucleus*, in spite of its containing some large cells, should not be considered as belonging to the nucleus of Deiters, as has been done by some authors. It should also be clearly kept apart from the *descending vestibular nucleus*, which is characterized by the presence of longitudinally running fibre bundles. Within the descending nucleus a more compact group of medium-sized cells, here called group *f*, is present in its ventro-lateral caudal part. The connexions of this group are discussed.

The *interstitial nucleus of the vestibular nerve* of Cajal appears to be an aberrant part of the nucleus of Deiters.

Some smaller cell groups, topographically related to the vestibular nuclei, but not belonging to them, are described.

The findings made are compared with those reported by other authors. Points of disagreement are discussed and the importance of detailed references to the topography in anatomical and physiological studies is stressed.

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EXPLANATION OF PLATE

Photomicrographs ($\times 125$) of transverse Thionine stained sections (Figs. 1–8) through the vestibular nuclei of the cat to show the characteristic architecture in the various nuclei. Fig. 9, a photomicrograph of a transverse Weil stained section ($\times 70$).

Fig. 1. From the rostral part of the lateral vestibular nucleus (cp. drawing 7 in Text-fig. 1). Giant cells interspersed with many medium-sized and small cells.

Fig. 2. From the caudal part of the lateral vestibular nucleus (cp. drawing 11 in Text-fig. 1). Chiefly giant cells, most of them larger than those present in the rostral part of the nucleus.

Fig. 3. The group '*l*' of medium-sized cells (within the broken line) on the lateral aspect of the lateral vestibular nucleus (cp. drawings 9–10 in Text-figs. 1 and 2). Lateral to the nucleus the fibres of the restiform body (*C.r.*) are seen.

Fig. 4. From the superior vestibular nucleus (cp. drawing 7 in Text-fig. 1).

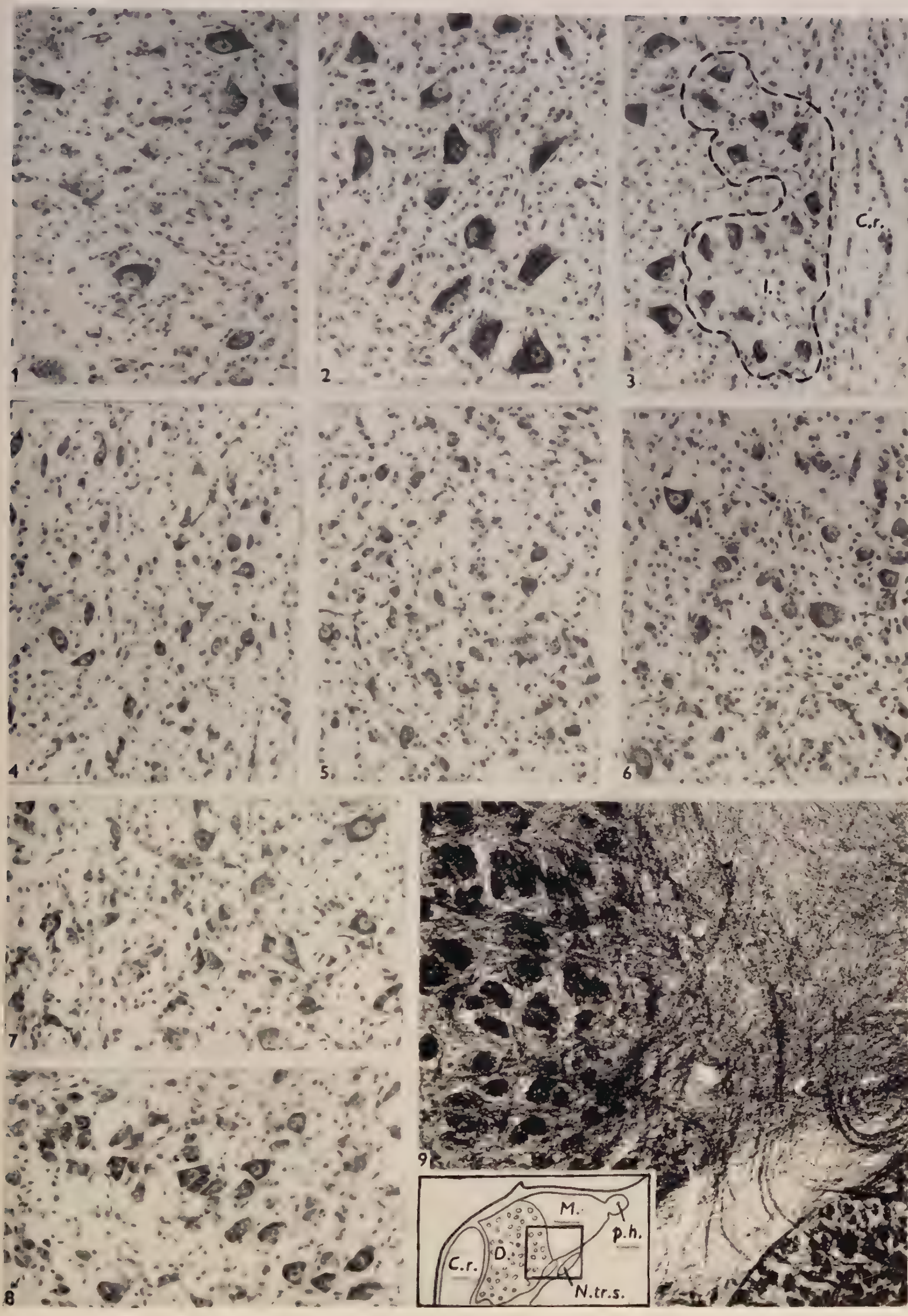
Fig. 5. From the rostral part of the medial vestibular nucleus (cp. drawings 7–9 in Text-fig. 1).

Fig. 6. From the middle levels of the medial vestibular nucleus (cp. drawings 11–13 in Text-fig. 1). Some large cells in addition to cell types present in the rest of the nucleus.

Fig. 7. From the descending vestibular nucleus (cp. drawings 11–15 in Text-fig. 1). Cells of different sizes, rather loosely arranged between the descending vestibular root fibres. One of the scattered larger cells is seen.

Fig. 8. Part of the fairly compact group, labelled '*f*', ventro-laterally in the caudal part of the descending nucleus (cp. drawings 16–21 in Text-fig. 1).

Fig. 9. In Weil stained sections the difference between the medial and descending nucleus is conspicuous. Abbreviations as in Text-fig. 1. Section from an adult cat ($\times 70$).



BRODAL AND POMPEIANO—VESTIBULAR NUCLEI

(*Facing p.* 454)

THE INNERVATION OF THE LIGAMENTS ATTACHED TO THE BODIES OF THE VERTEBRAE

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The object of this paper is to record the microscopic appearances of the nerve supply of the anterior and posterior longitudinal ligaments and of the annulus fibrosus as observed in sections of these structures taken from the thoracic region of the vertebral column. The material used was canine, and the sections were impregnated with silver.

The central connexions of the nerves will not be considered in detail. These connexions, however, help to explain the microscopic appearances, and a brief account of them as given by previous workers is included in the following survey of the literature.

LITERATURE

The innervation of the ligaments attached to the bodies of the vertebrae has been studied during the past 150 years, and although there are many discrepancies in the results recorded, one feature of particular interest stands out above all others. This feature is that the nerves of these ligaments not only arise from the spinal nerves but are also extensively derived from the peripheral part of the sympathetic system.

The earliest account of these nerves concern the anterior longitudinal ligament. Ribes & Beclard (1811), according to Rüdinger (1863), described pre-vertebral sympathetic nerves connecting the right and left sympathetic trunks in the cervical region, and Krause (1843) amplified this observation by describing similar connexions between the thoracic, lumbar and sacral ganglia of the opposite sides. Lobstein's (1823) observation that a very delicate filament from the first thoracic ganglion perforates the anterior longitudinal ligament and supplies the bone, was substantiated by Cruveilhier (1842), and was shown by him to be valid for all the ganglia of the sympathetic trunk.

The first mention of the nerves which are now known to supply the posterior longitudinal ligament was made by Purkinje (1845). He recorded that rich bundles of thin nerve fibres lie on the vertebrae and are directly related to the plexus of epidural venous sinuses. These fibres were regarded as having the character of the nerves of the sympathetic nervous system, and appeared to him to communicate with the sympathetic trunk through the intervertebral foramina. It is interesting that he ascribed to these fibres various sensory and reflex functions, and considered that strenuous efforts should be made to describe and illustrate their distribution.

This challenge was accepted by Luschka, who published a monograph on the nerves of the vertebral canal in 1850. He demonstrated a recurrent nerve passing medially through each intervertebral foramen and formed by a branch from a spinal

nerve arising just distal to the spinal ganglion and by a branch from a sympathetic ramus communicans. The nerve is described by Luschka as supplying the vertebrae, the venous sinuses and the cellular tissue in which the sinuses are imbedded, and he designated it the 'sinu-vertebral nerve'. He also recorded topographical variations of this nerve, but made no mention of the branches it gives to the posterior longitudinal ligament.

The nerves to the posterior ligament were first described by Rüdinger (1863), who published a detailed monograph on the sympathetic supply of the vertebral canal, spinal cord and brain. He considered that the posterior root of each spinal nerve contributes about 50 nerve fibres to the vertebral canal and that these are accompanied by some 100 sympathetic nerves, of which a few are Remak fibres, whereas the others and the spinal nerves have a double contour. This preponderance of sympathetic fibres is in harmony with Purkinje's original observations. Rüdinger was of the opinion that the vertebral column is more richly innervated than any other part of the body. He traced nerves to the tunica intima of the blood vessels of the vertebral canal, the dura mater, the periosteum, the bone, and ligamenta flava and, as he specially emphasized, the posterior longitudinal ligament and the intervertebral discs. Nerve fibres were found to pass transversely within the substance of the posterior longitudinal ligament throughout its length. These Rüdinger considered to be commissural fibres uniting the ganglia of the right and left sympathetic trunks on the posterior aspect of the vertebral bodies and comparable to the corresponding fibres discovered by Ribes & Beclard (1811), which unite the trunks on the anterior aspect of the column.

Hovelacque (1925) re-investigated the origin and distribution of the sinu-vertebral nerves, and confirmed their distribution to the dura mater, venous sinuses of the vertebral canal, the bodies and laminae of the vertebrae and the posterior longitudinal ligament. Jung & Brunschwig (1932) also corroborated many of the findings of previous workers. It is of special interest that they found free nerve terminations in the attachment of the anterior ligament to the annulus fibrosus, and the presence of unmyelinated fibres in the ligaments was considered to be in keeping with the view that these fibres are sympathetic. Also, Roofe (1940) found the annulus fibrosus and the posterior longitudinal ligament in the lumbar region to be innervated by fine unmyelinated nerve fibres, which terminated in naked nerve endings within these structures. He also described glomerulus-like terminations in the posterior longitudinal ligament. Ehrenhaft (1943) recorded the presence of large nerve bundles beneath the posterior longitudinal ligament and within the annulus fibrosus in a $5\frac{1}{2}$ month foetus. There were similar bundles within the annulus fibrosus. He also found that there are nerve fibres beneath the anterior longitudinal ligament, and that these give off fine branches, which accompany the vessels entering the disc substance.

Hirsch & Schajowicz (1953) found fairly large bundles of nerve fibres on the surface of the anterior and posterior ligaments. These give off thinner bundles, which accompany vessels and can be traced a short way into the outermost parts of the ligaments.

Pedersen, Blunck & Gardner (1956) made macro- and microscopical studies of the sinu-vertebral nerves in adult and foetal human material. They found that these

nerves had sympathetic and spinal components, and confirmed many other findings of previous workers.

Stilwell (1956) has given an extensive description of the nerve supply to the vertebral column and its associated structures in the monkey as revealed by a combination of myelin and intravital methylene blue stains. The nerves are branches of a segmentally arranged paravertebral plexus, which communicates with grey and white rami communicantes, with spinal and sympathetic ganglia and with the anterior and posterior primary rami of the spinal nerves. There is a one segment overlap in the innervation. The anterior longitudinal ligament is supplied by branches which arise directly from the sympathetic ganglia, and there is a plexus of nerve fibres throughout its substance but mainly near its deep surface. Fine nerve fibres are described as branching repeatedly to give delicate arborizing branches tapering to invisibility or ending in tiny enlargements, and fine beaded fibres continue along the vessels to form a network of vasomotor axons to the arterioles. The distribution of the nerves to the posterior longitudinal ligament is similar, but the nerves are derived from both somatic and visceral sources. The nerves of the annulus fibrosus are confined to a thin superficial lamina of loose connective tissue, and in this there are small blood vessels supplied by vasomotor nerves and a few beaded unmyelinated nerve fibres with free nerve endings.

MATERIAL AND METHOD

The writer has made many attempts to demonstrate nerves in the dense fibrous tissue of ligaments by silver impregnation. The best results have been obtained by the Gros–Schultze technique (Romeis, 1948), and the preparations described in this paper were made by this method.

The tissue was from the thoracic part of the vertebral column of dogs, and was fixed *in situ* by the injection of the arterial system with 20 % formalin (Analar) in normal saline (0·85 % sodium chloride) after the death of the animal. The material was kept in the fixative at room temperatures for periods of 5, 6 and 7 months. Portions of the anterior and posterior longitudinal ligaments along with the adjacent part of the annulus fibrosus were dissected out; washed overnight in running tap water, and cut into sections with the freezing microtome at 40, 50 and 100 μ. The plane of section was parallel to the free surface of the ligaments, and many of the deeper sections incorporated part of an annulus fibrosus.

OBSERVATIONS

Vertebral nervous plexus

A wide-meshed primary nervous plexus formed by ribbons of parallel nerve fibres surrounds the bodies of the vertebrae and is associated with the internal and external vertebral venous plexuses. These ribbons either divide or unite to form nodes so that there is a rich anastomosis of the ribbons (Pl. 2, fig. 9). Ribbons of finer fibres arise from the primary plexus, and penetrate the longitudinal ligaments (Pl. 2, fig. 8). Some also form a secondary plexus in relation to the antero-lateral aspects of the intervertebral discs (Pl. 3, fig. 12).

The primary plexus is regarded as receiving branches from the spinal nerves, sympathetic ganglia and rami communicantes as described by previous workers. The topographical details of the passage of such fibres from their origins to the plexus is not considered in this paper which is primarily concerned with the terminal distribution of the nerves.

Innervation of the posterior longitudinal ligament

Cords of a very delicate fibrillar net or reticulum have been found distributed among the collagenous fibre bundles of the posterior longitudinal ligament. In order to make as clear as possible the characters of this reticulum and its mode of origin and distribution, a specific specimen of the reticulum will be considered in detail.

This specimen is in the form of a cord, indicated by a pointer in Pl. 1, fig. 1, and arises from a bundle of nerve fibres and fibrils running parallel to a venule. Two nerve fibres emerge from the bundle and from these fibrils are continued into the fibrillar net of the cord. Here at its origin the net encloses a flat nucleus which on surface view appears four sided with rounded corners. At first the cord runs away from the nerve bundle and at right angles to the collagenous fibres of the ligament, but gradually it changes its direction until it is parallel to them. It finally divides into two cords, and in the node formed by this division there is again a four-sided nucleus, marked by a pointer in Pl. 1, fig. 2. This nucleus is related to delicate, fenestrated cytoplasm, which is not visible in the photograph.

The cord and its subdivisions are imbedded among the collagenous fibre bundles of the section, which is 50 μ thick, but the reticulum can be seen with great clearness, since the collagenous bundles are not impregnated and are transparent. These bundles can be observed, however, by diminishing the aperture of the iris diaphragm and using the nuclei of the fibrocytes of the ligament as a guide in focusing the successive planes of the section.

Of the two divisions of the cord one runs to the left parallel to the parent nerve bundle (Pl. 1, fig. 2). This cord of the reticulum varies in width along its course. Finally, it tapers and disappears within the section. The other division runs to the right and likewise tapers to invisibility.

A similar cord of the reticulum also in the posterior longitudinal ligament, but from another section and as seen with the oil immersion objective, is shown in Pl. 1, fig. 3. This cord also runs parallel to the collagenous fibre bundles, and is imbedded among them. It arises by the division of a parent cord, and in the photograph a cleft can be seen within it to the left of an oval, elongated nucleus. This nucleus is heavily impregnated, but when more lightly impregnated such nuclei, which characterize the cords, have the appearance of ground glass and show small granules and prominent nucleoli. They are elongated into rods in the narrower parts of the cords. These nuclei of the cords have been termed Schwann nuclei, and are thereby distinguished from the angular nuclei at the nodes produced by the branching of the cords, which have been termed 'nuclei of interstitial cells'. The impregnated reticulum is composed of fibrils of varied width and density of impregnation. The fibrils form a net, the meshes of which vary greatly in size and shape. They are varicose and at the sites of the varicosities they may change their direction. The varicosities are spherical or spindle-shaped, and are usually more

deeply impregnated with silver than the fibrils themselves. The openings of the net in some places are as clear as the background surrounding the reticulum, but in others they are occluded by a faintly impregnated membrane.

It is to be noted that in this preparation there is no sign of a nerve fibre within the cords. The nuclei of the reticulum cannot, therefore, be regarded as the nuclei of Schwann cells forming the neurolemma of a nerve fibre. Nor for the same reason can the reticulum be considered as an artefact derived from the myelin sheath of a nerve fibre, and this view is still further supported by the presence of a nucleus within a cord of the reticulum at the site of its division.

A fragment of the reticulum from the extreme left part of the field shown in Pl. 1, fig. 2, and lying below the longitudinally running cord is shown at a higher magnification in Pl. 1, fig. 4. This fragment is a web, triangular in shape and composed of fibrils and apparently isolated granules lying in contact with the collagenous fibres of the ligament.

The cords of the reticulum in the posterior longitudinal ligament have been observed also to arise from fibrillated cytoplasm within the ribbons of parallel nerve fibres derived from the primary vertebral nervous plexus. This cytoplasm is associated with nuclei resembling the nuclei of the interstitial cells.

The origin of a cord of the reticulum from the region of the attachment of the posterior longitudinal ligament to an intervertebral disc is shown in Pl. 2, fig. 6. One of the fibres in a ribbon gives off a branch which runs downwards in the figure. This branch is a fine nerve fibre which accompanies a flattened and relatively broad cord of faintly fibrillated cytoplasm also emerging from the ribbon. The breadth of this cord is indicated by the pointers in the lower part of the figure. Its fibrils are regarded as branches of the nerve fibres of the ribbon. More peripherally the fine nerve fibre and the fibrils shown in the photograph are replaced by the typically varicose fibrils of the nervous terminal reticulum. This particular cord of the reticulum was not traced into the posterior longitudinal ligament, and is reproduced to show the mode of origin of a cord.

There are other terminal nerve formations in the posterior longitudinal ligament. Fibres arising from the ribbons may run singly or in groups through the ligament and parallel to the collagenous bundles. Two of these fibres are reproduced in Pl. 1, fig. 5. These fibres unravel into fibrils and reform again. Finally, one alone persists and is accompanied by a delicate reticulum representing the continuation of the other.

Pl. 2, fig. 7 depicts a complex nerve ending. The edge of a ribbon of nerve fibres imbedded in the posterior longitudinal ligament and running transversely through it is shown in the upper part of the figure. One of the nerve fibres gives off a branch as thick as itself. This branch divides into fibres of which some re-enter the ribbon, and some extend outwards from it to end in nucleated and fenestrated cytoplasm as delicate fibrils.

Innervation of the anterior longitudinal ligament

Ribbons of nerve fibres comparable to those in the posterior ligament also run through the anterior ligament. Pl. 2, fig. 8 shows such a ribbon as it winds its way transversely between the collagenous fibre bundles to which it is closely applied.

It consists of thick and thin nerve fibres and delicate fibrils and amongst these are nuclei of the interstitial cell type. These are accompanied by very faintly impregnated cytoplasm, which here and there is in the form of indefinite processes giving the general appearance of a ganglion cell of the multipolar, autonomic type. Cells with processes containing neurofibrils have, however, not been observed. One of the thick fibres gives off two processes, which fade into the cytoplasm in the vicinity suggesting a synaptic formation. These processes are indicated by a pointer in Pl. 2, fig. 8, and as seen with the oil immersion objective, in Pl. 2, fig. 11. Very fine nerve fibres and fibrils pass from the ribbon into the surrounding ligament, and single nerve fibres have been observed coursing through the ligament for long stretches. These may end by tapering.

The presence of fibrils and of cytoplasm with nuclei of the interstitial cell type in the nerve ribbons of the anterior longitudinal indicates that the cords of the reticulum found in the posterior ligament are also present in the anterior. They are either not so plentiful, however, or have not been so successfully impregnated, for only three specimens of these cords have been found in the sections prepared. One of these is reproduced in Pl. 2, fig. 10.

Innervation of the surface layers of the annulus fibrosus

The vertebral nervous and venous plexuses are well developed at the surface of the annulus fibrosus antero-laterally, and extend here between the superficial layers of the annulus immediately lateral to the anterior longitudinal ligament. The general picture resembles that found by Rossi (1950) in the synovial membrane of the shoulder joint, and by Sunder-Plassmann & Daubenspeck (1938) in the synovial membrane of the knee joint. The vertebral nervous plexus is of the secondary type, and consists of fine branching nerve fibres and fibrils with fenestrated cytoplasm and nuclei of the interstitial cell type at the nodes of the plexus (Pl. 3, fig. 12).

The nerve fibres present frequent unravellings into fibrils as shown by the pointer in Pl. 3, fig. 15. The unravelling of the fibrils of the nerve fibre is similar to the unravelling of the collagenous fibre bundles of the plantaris tendon produced by stretching it locally into a broader band.

This unravelling may take place at the site of origin of a cord of the nervous terminal reticulum to give rise to the fibrils of the reticulum. Thus, in the upper part of the field shown in Pl. 3, fig. 14, there is a ribbon of nerve fibres and fibrils running from the upper margin to the right margin of the figure. From this ribbon a cord of the reticulum containing an interstitial nucleus extends downwards. To the left of the origin of this cord there are four nerve fibres in the ribbon, but only two of these pass into the part (not in focus) of the ribbon to the right. The other two fibres split into fibrils continuous with the fibrillar net of the reticulum. The site of the division of one of these fibres is indicated by the pointer. The cords of the nervous terminal reticulum so formed extend between the layers of the collagenous fibre bundles at the surface of the annulus fibrosus. They differ from those of the posterior longitudinal ligament in that the fibrils are in general thicker and seldom varicose (Pl. 3, fig. 13).

The reticulum at the surface of the annulus fibrosus not only arises from the fine

fibres of the secondary plexus, but is also connected with thick, myelinated fibres. Thus cords of the reticulum may be seen extending upwards from the transverse ribbon of thin nerve fibres shown in Pl. 3, fig. 16. One of these cords becomes continuous with a reticulum adjacent to a branch of a heavily myelinated fibre. This branch, seen projecting convexly down from the upper border of the figure, gives off a slender nerve fibre which merges with one of the cords. There is thus some evidence that the reticulum may be derived from nerves of two different types, and, therefore, possibly of different origins.

A very elaborately branched nerve fibre with fibrillar expansions of varying width has also been observed in this region. It lies in contact with the secondary nerve plexus (Pl. 3, fig. 17).

DISCUSSION

The reticulum in the anterior and posterior longitudinal ligaments and between the surface layers of the annulus fibrosus is in the form of cords, and is the terminal distribution of a plexus of nerve fibres. As seen with the light microscope these cords are not continuous with any more peripheral structure, but it is possible that an improved silver technique or the use of the electron microscope may show that such connexions exist.

The reticulum is a net of varicose fibrils continuous centrally with nerve fibres. The net is associated with cytoplasm most evident in silver preparations at the sites of division of the cords, and at such nodes there is often a flattened nucleus, which on surface view appears to be three or four sided with rounded corners. These nuclei with the surrounding cytoplasm form the so-called interstitial cells, although clearly defined cell processes are not visible. The nuclei of the cords are oval or rod-shaped and in the literature have been termed Schwann nuclei, thus indicating that they belong to cytoplasm associated with the fibrillar net as the neurolemma is associated with a nerve fibre.

This reticulum is the nervous terminal reticulum of Stöhr and Reiser. It has been subdivided into parts and described under several names by different authors, and an analysis of these descriptions and nomenclatures has been given by Stöhr (1954). Reiser, for example, has subdivided the nervous terminal reticulum into a central part, the pre-terminal net as in Pl. 1, fig. 3, and a peripheral part, the terminal reticulum proper as in Pl. 1, fig. 4. Of the various terms available the designation nervous terminal reticulum is particularly suitable in that it describes the morphology of the structure and makes no reference to its incompletely understood functions.

The reticulum has been regarded as an artefact produced by formalin fixation. This may be said of the fibrillar structure of nervous cytoplasm in general as revealed by various techniques, but the fibrils may, nevertheless, be the expression in the fixed state of a parallel orientation of molecules in the living cytoplasm. The theory that the reticulum is a formalin artefact has, however, been rejected by Jabonero (1954) on the ground that the reticulum is revealed without the use of formalin by supravital staining with methylene blue. Zander & Weddell (1951), however, suggest that the pre-terminal net of Reiser, that is the more central part of the nervous terminal reticulum, is the cytoplasm of Schwann cells overstained with

methylene blue. The cords of the reticulum described above may, however, run for relatively long distances and contain not an axon but a fibrillar net continuous centrally with fine nerve fibres. These nerve fibres may themselves show localized fibrillar expansions similar in structure to parts of the nervous terminal reticulum. Further, there may be a nucleus at the site of branching of the cords of the reticulum. These appearances have led me to the conclusion that a cord of the reticulum is not an artefact derived from the myelin sheath or from the neurolemma of a nerve fibre, but that it consists of a fibrillar net associated with nucleated cytoplasm. Such a conclusion is in accordance not with the concept that the reticulum is an artefact derived from a neurolemmal sheath, but with the view that it has a composite structure embodying both neurolemmal sheath and nerve fibrils. Nevertheless, the exact morphological and functional significance of this sheath and of the interstitial cells requires further study.

The nervous terminal reticulum supplies unstriped muscle and glands throughout the body and is generally regarded as motor in function. The reticulum distributed to the ligaments attached to the bodies of the vertebrae is thus of special interest in that it may leave the blood vessels to be imbedded among the collagenous fibre bundles, where there is no gland tissue and no unstriped muscle. Kiss (1956) also has recently shown that collagenous fibres are supplied by the terminal reticulum. These observations raise important questions concerning its function. The single nerve fibres which end by tapering or by unravelling into fibrils and the thick, elaborately branched nerve fibres are probably receptor in character. It is interesting that the nerve ending shown in Pl. 3, fig. 17 resembles the receptor endings associated with the unstriped muscle of the human lung (Stöhr, 1954).

SUMMARY

1. The ligaments attached to the bodies of the thoracic vertebrae in the dog receive their nerve supply from a plexus of nerve fibres associated with the internal and external vertebral venous plexuses.

2. From this plexus ribbons of fine parallel nerve fibres arise of which some traverse the anterior and posterior longitudinal ligaments, and some form a secondary plexus at the surface of the annulus fibrosus lateral to the anterior longitudinal ligament.

3. The posterior longitudinal ligament and to a lesser extent the anterior longitudinal ligament are supplied by the nervous terminal reticulum. Cords of a modified form of this reticulum arising from the secondary vertebral nervous plexus supply the superficial layers of the annulus fibrosus antero-laterally.

4. Longitudinally running nerve fibres which may taper or end in a fine reticulum and elaborately branched nerve terminations are also found in these ligaments.

I wish to thank Prof. Stöhr, jr., Director of the Anatomical Institute of the University of Bonn am Rhein, for having corroborated the presence of vegetative nerve formations in the preparations described in this paper, and also for very many other kindnesses shown to me during the past 22 years.

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Finally, I record here my gratitude to the late Prof. David Waterston and to Prof. Robert Walmsley of the Department of Anatomy, St Salvator's College, University of St Andrews, who each in turn have greatly encouraged me in the study of the peripheral nervous system and have given me every laboratory facility at their disposal. I wish to thank Prof. Walmsley in particular for helping me in the preparation of this paper.

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EXPLANATION OF PLATES

All the figures have been made from eight sections of the anterior and posterior longitudinal ligaments and annulus fibrosus cut parallel to the free surfaces of the longitudinal ligaments at 40, 50 and 100 μ, and impregnated with silver by the Gros-Schultze technique. Figs. 7, 8 and 12 are from drawings made with the aid of Abbe's drawing apparatus. The remaining figures are untouched photomicrographs.

Plate 1

Fig. 1. Posterior longitudinal ligament. A bundle of nerve fibres and fibrils lies below and parallel to a venule in the upper part of the figure. A cord of the nervous terminal reticulum extends downwards from this bundle, and is marked by the pointer. This cord is imbedded among the collagenous fibre bundles of the section. Thickness of section, 50 μ. ($\times$429 approx.)

Fig. 2. Posterior longitudinal ligament. This shows a continuation of the cord in the previous figure. It divides at the site of the nucleus marked by the pointer. One of the divisions is out of focus, and the other runs to the left imbedded among and parallel to the collagenous fibre bundles of the ligament. Thickness of section, 50 μ. ($\times$550 approx.)

Fig. 3. Posterior longitudinal ligament. A cord of the nervous terminal reticulum similar to the cord in the previous figure, but from another section and at a higher magnification. Thickness of section, 100 μ. ($\times$1762 approx.)

Fig. 4. Posterior longitudinal ligament. A fragment of the nervous terminal reticulum also shown at the left of fig. 2 below the longitudinally running cord. Thickness of section, 50 μ. ($\times$1769 approx.)

Fig. 5. Posterior longitudinal ligament. Nerve fibres running parallel to the collagenous fibre bundles of the ligament. They unravel into fibrils and reform along their course. Thickness of section, 50 μ. ($\times$1762 approx.)

Plate 2

Fig. 6. Posterior longitudinal ligament at the level of an intervertebral disc. The edge of a ribbon of nerve fibres is seen at the top right-hand corner of the figure. A fibrillated cytoplasmic band accompanied by a tapering nerve fibre runs downwards from the ribbon. Its breadth is indicated by the pointers in the lower part of the figure. This band is the central end of a cord of the nervous terminal reticulum. Thickness of section, 50 μ. ($\times$ 1796 approx.)

Fig. 7. Posterior longitudinal ligament. The edge of a ribbon of nerve fibres imbedded in the ligament and crossing it transversely is shown in the upper part of the field. The lowest fibre gives off a branch which in turn gives smaller branches, some of which re-enter the ribbon and some end in nucleated cytoplasm below the ribbon. Thickness of section, 100 μ. ($\times$832 approx.)

Fig. 8. Anterior longitudinal ligament. A ribbon of nerve fibres and fibrils runs transversely through the ligament. There are nuclei accompanied by faintly impregnated cytoplasm throughout the ribbon. One of the fibres of the ribbon shows lateral extensions into the cytoplasm at the site marked by the pointer along the left margin of the figure. Thickness of section, 100 μ. ($\times$621 approx.)

Fig. 9. Nodes of the primary vertebral nervous plexus adjacent to the annulus fibrosus and lateral to the anterior longitudinal ligament. ($\times$722 approx.)

Fig. 10. Anterior longitudinal ligament. A cord of the nervous terminal reticulum imbedded among the collagenous fibre bundles of the section. Thickness of section, 40 μ. ($\times$1866 approx.)

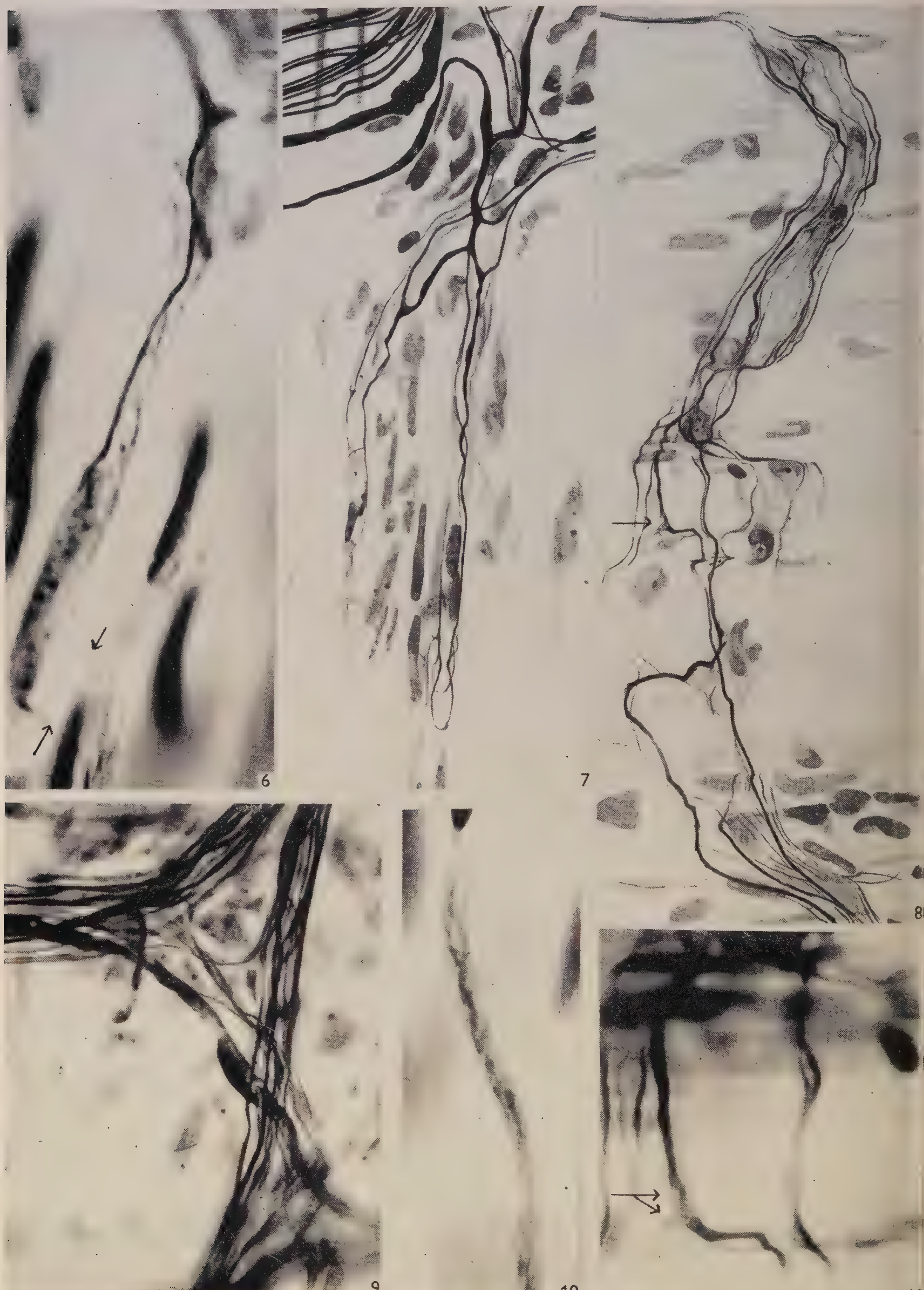
Fig. 11. Microphotograph of the lateral extensions of the nerve fibre also marked by a pointer in fig. 8. Thickness of section, 100 μ. ($\times$1912 approx.)

Plate 3

Fig. 12. The secondary vertebral nervous plexus adjacent to the antero-lateral surface of the annulus fibrosus. It consists of fine, branching nerve fibres and fibrils with fenestrated cytoplasm and nuclei of the angular, interstitial cell type. The cords of fine nerve fibres and fibrils to the right are between the surface layers of the annulus fibrosus. Thickness of section, 100 μ. ($\times$745 approx.)

MULLIGAN—LIGAMENTS ATTACHED TO BODIES OF THE VERTEBRAE

(*Facing p.* 464)



MULLIGAN—Ligaments attached to bodies of the vertebrae

MULLIGAN—LIGAMENTS ATTACHED TO BODIES OF THE VERTEBRAE

Fig. 13. Cord of the nervous terminal reticulum imbedded between the antero-lateral surface layers of the annulus fibrosus. Thickness of section, 100 μ. ($\times$ 1573 approx.)

Fig. 14. Part of the secondary nervous plexus adjacent to the antero-lateral surface of the annulus fibrosus. A ribbon of nerve fibres crosses the upper part of the field running from the upper to the right margin of the figure. A cord of the terminal reticulum containing an interstitial cell nucleus runs vertically down from the ribbon. A nerve fibre divides into fibrils at the site indicated by the pointer, and contributes to the formation of the reticulum. Thickness of section, 100 μ. ($\times$ 1443 approx.)

Fig. 15. A nerve fibre in the secondary vertebral nervous plexus unravels into its constituent fibrils at the site marked by the pointer. Thickness of section, 100 μ. ($\times$ 1578 approx.)

Fig. 16. From the antero-lateral surface of the annulus fibrosus. The nervous terminal reticulum stretches between a ribbon of nerve fibres in the lower part of the figure and a nerve fibre arching convexly downwards from the upper margin of the figure. This nerve fibre, which arises from a medullated nerve fibre (not in the photograph), gives off a very fine fibre to the reticulum. Thickness of section, 100 μ. ($\times$ 953 approx.)

Fig. 17. From the antero-lateral surface of the annulus fibrosus. A branched nerve fibre with irregular fibrillar expansions of its branches lies in direct contact with the secondary vertebral nervous plexus. Thickness of section, 100 μ. ($\times$ 457 approx.)

CONCERNING THE GANGLIFORM ENLARGEMENT ('PSEUDOGANGLION') ON THE NERVE TO THE TERES MINOR MUSCLE

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An oval swelling on the nerve to the teres minor muscle is mentioned in a number of standard anatomical texts and is variously termed a 'gangliform enlargement' (Schaeffer, 1942; Brash, 1949) or 'pseudoganglion' (Johnston & Whillis, 1949). A gangliform enlargement is described also near the terminal bifurcation of the median nerve (Jones, 1953), at the termination of the dorsal interosseous nerve of the forearm and on the lateral terminal branch of the anterior tibial nerve. The swellings on the latter three nerves are in close contact with ligamentous, tendinous or bony structures; in the case of the swelling on the nerve to the teres minor no similar relationship is apparent at first sight nor is any such relationship referred to in the above-mentioned texts.

The present work was undertaken to study the form and relations of this swelling and also some aspects of its microscopic structure.

MATERIALS AND METHODS

(*a*) *Macroscopic findings* are based on thirty-six dissections; thirty of these were in adult dissecting room cadavers and the remainder in adult autopsy cases.

(*b*) *Microscopic examination* was directed towards:

(i) Ascertaining the types of cells present in the enlargements. Five specimens from autopsy cases were examined, technique consisting of fixation in Bouin's solution or formalin, embedding in paraffin and staining with hematoxylin and eosin.

(ii) Comparing the amount of connective tissue in the enlargements with that in the nerves immediately proximal to the enlargements. The relative cross-sectional area, in these two regions, of the epineurium, perineurium and nerve fibre fasciculi (i.e. endoneurium plus nerve fibres) was determined by making projection drawings of microscopic sections on millimetre squared paper (Sunderland & Bradley (1949), slightly modified); a total of four specimens was used. Histological technique was as in (i).

With regard to the endoneurium itself, a better indication of the amount present in an enlargement, as compared with that in the nerve proximal to the enlargement, was obtained from azan-stained sections. Sections from the two regions were examined simultaneously by means of a Bausch & Lomb comparison eyepiece.

OBSERVATIONS

(*a*) *Macroscopic findings* (Fig. 1, Table 1)

In outline the findings were as follows:

(i) An elongated, fusiform swelling was found along the course of the nerve in twenty-one of the thirty-six specimens dissected.

(ii) In five specimens the nerve to the teres minor was found to be distinctly broad but the broadening was not fusiform in shape.

(iii) In the remaining ten cases neither fusiform swelling nor any other form of broadening of the nerve was observed.

Table 1. *Data from dissections of the nerve to the teres minor*

	No. of cases	Relationship of nerve to teres minor to long head of triceps: Curving round	Lying alongside
Fusiform swelling of nerve present	21	20	1
Broadening of nerve present, not fusiform in shape	5	4	1
Neither fusiform swelling nor other form of broadening of nerve present*	10	4	7

* In one of these cases two branches proceeded to the teres minor, one lying alongside and the other curving round the long head of the triceps. Each of these is entered separately in the two right-hand columns of the table, hence the discrepancy in the figures.

(i) *The fusiform swelling*, when present, was approximately 12–20 mm. long and 2·5–4·0 mm. wide; it was flattened to a degree varying from case to case. Its commencement along the course of the nerve was gradual and it terminated, after gradually narrowing again, by giving off branches to the teres minor. In no case did the swelling take the form of a small, sharply circumscribed enlargement such as illustrated by Brash (1949, fig. 46), Jones (1953, fig. 280) and Mitchell & Patterson (1954, fig. 74). In one instance the narrowing distal to the fusiform swelling was succeeded by a second slight swelling from which arose the terminal branches supplying the teres minor. In one other case the gangliform enlargement was seen to be split longitudinally, but unequally, into two; each part, after narrowing, gave branches to the teres minor.

In a small number of cases the fusiform enlargement involved not only the nerve to the teres minor itself but the whole of the posterior division of the circumflex nerve. In these cases muscular branches to the deltoid and also a cutaneous branch were seen to come off the swelling.

Relations. The nerve to the teres minor (or the posterior branch of the circumflex nerve) was seen to arise from the superior aspect of the circumflex nerve deep in the quadrilateral space. In twenty of the twenty-one cases in which the fusiform swelling was present, the nerve at the site of the swelling performed a distinct curve round the lateral aspect of the upper part of the long head of the triceps, being closely applied to it; in this region the long head of the triceps is still distinctly tendinous on its surface. One of the terminal branches of the nerve to the teres minor frequently continued the curvature of the nerve as shown in Fig. 1. The capsule of the shoulder joint was related to the outer convex aspect of the nerve, but the relationship was

not nearly as intimate as that between the nerve and the tendon of the long head of the triceps; a variable quantity of fatty loose connective tissue was found between the nerve and the joint capsule. The relationship of the nerve to the surgical neck of the humerus was also not close.

In the one exceptional case the fusiform swelling was observed not to curve round the tendon of the long head of the triceps but to lie alongside its lateral aspect.

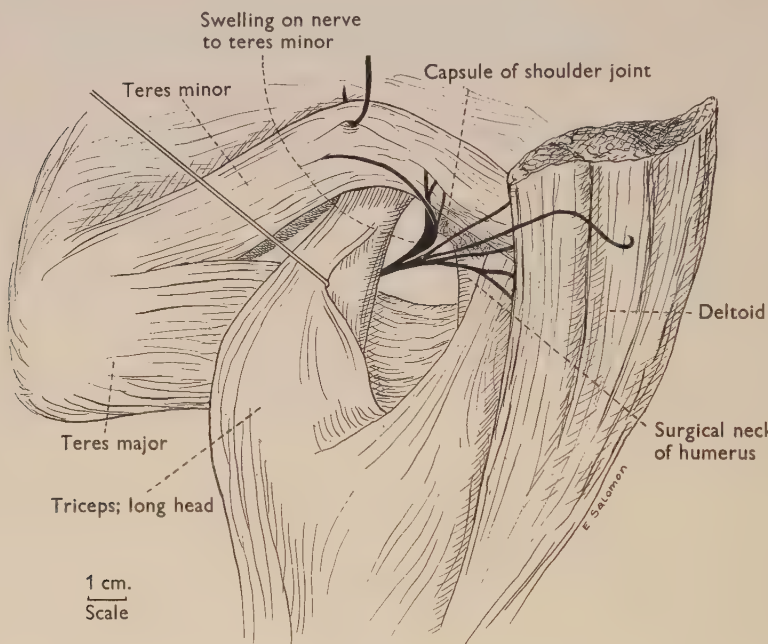

Fig. 1. Dissection showing a typical 'gangliform' enlargement on the nerve to the teres minor. The nerve at the site of the enlargement curved round the lateral aspect of the upper part of the long head of the triceps and was closely applied to it. The long head of the triceps has here been drawn medialwards.

(ii) In the five cases in which a *broadening—not fusiform in shape*—was present, its form, in four instances, was such that the nerve to the teres minor expanded as it passed dorsally, but did not narrow again before giving its terminal branches to the muscle. In the fifth case the nerve was broad at its origin and narrowed before entering the teres minor.

The relationship of these broadenings to the long head of the triceps is indicated in Table 1.

(iii) In the ten cases in which *no swelling was evident* it was found that in six instances the nerve passed supero-laterally directly to the teres minor, lying alongside the tendon of the long head of the triceps but not curving round it. In three instances it did curve round the tendon as described above.

In the remaining case two nerves to the teres minor were present; one of these lay alongside the tendon of the long head of the triceps while the other curved round the tendon.

(*b*) *Microscopic findings*

(i) The types of cells seen in sections of the enlargement were the same as those seen in peripheral nerves generally and in sections of the nerve to the teres minor proximal to an enlargement.

No ganglion cells were observed.

(ii) The absolute cross-sectional area of epineurium, perineurium and nerve fibre fasciculi (i.e. endoneurium plus nerve fibres) was, in each instance, markedly greater in the gangliform enlargement than in the nerve proximal to the enlargement. The percentage of the total area occupied by each of these components was different in the gangliform enlargement from that in the nerve proximal to the enlargement, but the number of specimens examined was too small for adequate statistical analysis.

The azan-stained sections showed that the quantity of intrafascicular collagenous material was greater in the enlargements than in the nerves proximal to the enlargements.

DISCUSSION

It would appear from the microscopic examination of the swellings on the nerve to the teres minor that they are due to the presence of relatively more connective tissue than is present in the nerves proximal to the swellings.

Dissection revealed that frequently the nerve curved round the lateral aspect of the upper, tendinous part of the long head of the triceps and was intimately applied to it. It may be seen from Table 1 that of the twenty-eight cases in which the nerve was in this fashion related to the triceps, twenty-four showed either a fusiform enlargement or a broadening, not fusiform in shape. It is possible that this intimate contact with the long head of the triceps bears a causal relationship to the presence of the swelling on the nerve; during muscular activity it is likely that friction occurs between the nerve and the tendon and that pressure is exerted on the nerve.

Why in a small number of instances the same intimate relationship to the tendon of the long head of the triceps was unaccompanied by a thickening or widening of the nerve may only be surmised. It is possible that individual differences exist in the degree of connective tissue reactions to mechanical forces. Such individual differences may explain also those two cases in which a widening of the nerve was present, but in which the nerve did not curve round the tendon of the long head of the triceps (Table 1). The nerves in these two cases simply lay alongside the tendon, and it is possible that a lesser degree of friction was sufficient to evoke a connective tissue hyperplasia.

SUMMARY

In thirty-six dissections of the nerve to the teres minor a fusiform swelling along its course was found in twenty-one instances and a broadening of the nerve, not fusiform in shape, in five instances. In twenty-four of these twenty-six cases the nerve, at the site of its enlargement, curved round the lateral aspect of the upper, tendinous part of the long head of the triceps, being closely applied to it.

On histological examination it was found that the cell types present in fusiform enlargements were the same as those seen in peripheral nerves generally. No ganglion cells were observed. The amount of connective tissue in enlargements was

considerably greater than in nerves proximal to the enlargements, the increase affecting epineurium, perineurium and endoneurium.

The possibility is discussed of the intimate contact between the nerve and the long head of the triceps bearing a causal relationship to the development of this increased amount of connective tissue, and thus to the presence of an enlargement along the course of the nerve.

The author wishes to thank Prof. M. Ickowicz for advice and assistance in connexion with the histological aspects of this work; Prof. H. Ungar and his staff for facilities provided in obtaining post mortem specimens; Mrs E. Salomon for the drawing of Fig. 1 and for the projection drawings; Miss T. Daskal, Mr I. Hayoun and Mr A. Welner for technical assistance.

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THE EPIBRANCHIAL PLACODE OF THE VAGUS NERVE IN THE SHEEP

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INTRODUCTION

The presence of a single epibranchial placode in relation to the distal ganglion of each of the three caudal visceral arch nerves, VII, IX and X, has been described in the ammocoete (Kupffer, 1891), and in numerous fish and amphibian embryos (Brachet, 1907; Knouff, 1927, 1935; Kostir, 1924; Landacre, 1908, 1910*b*, 1912, 1916, 1931, 1933; Reed, 1916). At each placodal thickening an intense mitotic activity produces a more or less compact cellular mass which detaches and migrates to the neural crest rudiment of the ganglion. The subsequent fate of these placodal cells may be traced without difficulty since they differ from mesenchymal and crest cells in size, pigmentation, yolk content and staining affinity. There is broad agreement from the study of normal development that the placodal cells differentiate into neuroblasts and sheath cells, and this conclusion has been substantiated by the results of the extirpation and transplantation experiments of Stone (1922). It is to Landacre (1910*a*, 1911, 1914, 1920), however, that we owe the concept of the functional specificity of the several types of placodal thickening on the vertebrate head.

In mammals the relationship between the placodes and the developing ganglia is much less easily interpreted, and even in the same species contradictory opinions have been expressed on the participation of the placodes in ganglion formation. Thus Adelmann (1925) failed to find any evidence of cellular migration in the rat and turned to the original interpretation of the epibranchial placodes as transient relics of sense organs which had fallen into disuse in the mammal (Froriep, 1885). Landacre (1932), however, was firmly convinced of the neurogenic activity of the facial epibranchial placode in the rat. Later investigators, notably Campenhout (1935, 1936, 1937, 1948), Coërs (1946) and Halley (1955), have shown that the epibranchial placodes contribute to the mixed ganglia in the pig, human, rabbit and cat.

The confusion in the literature is due to two factors: first, the greater difficulty of following migrating ectodermal cells in the mammal owing to the lack of distinguishing features, and, secondly, the marked difference in the character of placodal activity. In lower vertebrates the detachment and migration of cells occurs on a massive scale, whereas in mammals small spurs detach intermittently and are more easily overlooked.

The aim of this investigation has been to determine the extent to which the epibranchial placodes contribute to the developing geniculate, petrosal and nodose ganglia. In this attempt, counts of the mitotic figures in the placodal epithelium, of the number of placodal spurs and of the total number of migrating cells have been used as indications of proliferative activity. This quantitative approach has

previously been employed in a study of the trigeminal placode (Batten, 1957) and has the advantage of yielding new information on the changing pattern of proliferation during the active life of the placode. The epibranchial placode of the vagus nerve is the best developed of the series and has been chosen for this report because it provides clear evidence of the transformation of placodal cells into neuroblasts.

MATERIALS AND METHODS

The material used consists of 100 sheep embryos ranging from the 14-somite to the 16 mm. stage (16–29 days). An original series of twenty embryos, which was kindly presented by Prof. E. C. Amoroso, has been augmented by specimens collected from the Bristol abattoirs during the past three seasons. The writer has also been privileged to examine a further collection of stained embryos of known age through the courtesy of Prof. J. D. Boyd. Bouin's fluid has been routinely used for fixing specimens collected locally and after paraffin embedding these have been cut serially at either 7 or 10 μ and stained with Ehrlich or Weigert haematoxylin and eosin. Graphic reconstructions of the head region have been prepared from enlarged tracings of alternate sections of selected embryos which were cut serially at 10 μ for this purpose.

OBSERVATIONS

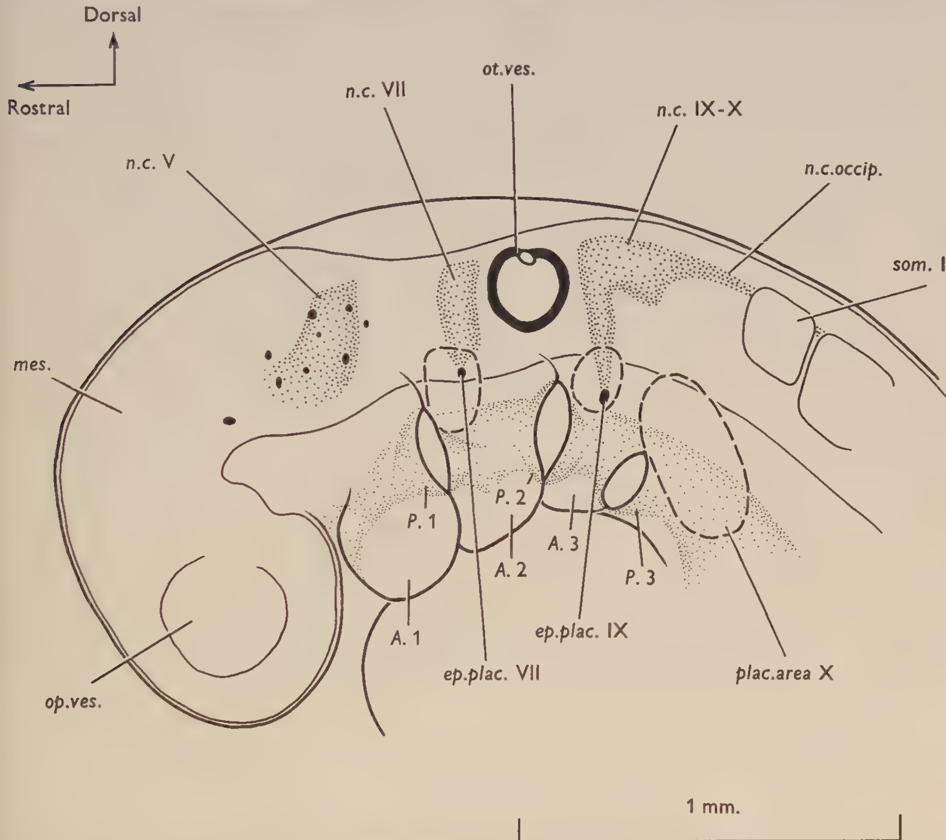
14–38-*somite embryos*, 16–21 *days*

Early development of the vago-glossopharyngeal neural crest. The earliest indication of the vago-glossopharyngeal neural crest is a short mass of diffusely arranged cells lying close against the hind-brain in the 14-somite embryo. About the 26- to 30-somite stage, the cranial portion of this common mass grows down into the second arch to establish the anlage of the IX nerve; this ends short of a broad ectodermal thickening in which the glossopharyngeal epibranchial placode will later appear (Text-fig. 1). The remaining part of the crest includes a short lobe, which presages the outgrowth of an independent vagal rudiment, and a narrow caudal extension representing the occipital neural crest. Dorsal to the broad closing plate of the third pharyngeal pouch the ectoderm is thickened over an extensive epibranchial area within which the placode of the vagus nerve will shortly differentiate.

With the outgrowth of the vagal anlage, about the 32-somite stage, the crest cells become segregated into two groups: a diffuse jugular group which remains broadly continuous with the glossopharyngeal nerve; and a distal group forming the nodose ganglion (Text-fig. 2). Within this ganglion the slight enlargement of the nuclei of crest cells marks the beginning of their differentiation into young preneuroblasts. In most embryos with 32 somites the petrosal and nodose ganglia are entirely separate, but occasionally a connecting loop of crest cells may persist for a short time, as in the embryo reconstructed. The hypoglossal nerve is represented by three groups of short fibrous outgrowths from the ventro-lateral surface of the hind-brain.

Epibranchial placode. Over the dorsal part of the fourth arch the ectoderm is markedly thickened in a slightly depressed area, the approximate limits of which are shown by the broken line in Text-fig. 2. Peripherally this thickened placodal ectoderm shows a gradual transition to common ectoderm (Pl. 1, fig. 1). The onset of proliferative activity within the placode appears to coincide with the arrival of

the nodose ganglion beneath it during the 26- to 33-somite stages. In most embryos the placode contains a minimum of six mitotic figures, but there is some individual variation in the time of appearance of the first spur (Table 1). At several points the deep surface of the epithelium becomes raised into minute papillae or short cytoplasmic spurs containing up to four nuclei. Over the majority of these papillae the basement membrane is still intact when examined under oil immersion, but later development reveals that they are sites of an impending detachment of placodal cells. In a few examples, however, the basement membrane has already been

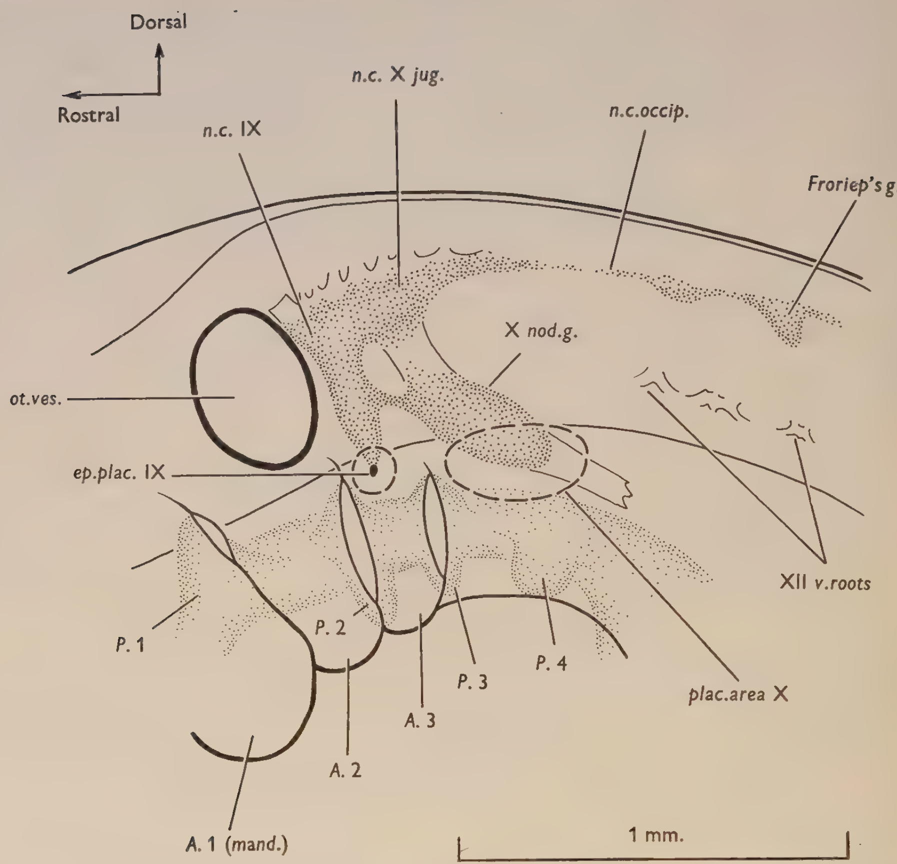


Text-fig. 1. Profile reconstruction of the left side of a 26-somite sheep embryo. The epibranchial thickenings are indicated by a broken line. Magnification ×50. See list of abbreviations, p. 487, for this and subsequent figs.

ruptured and one or two nuclei appear to be streaming towards the ganglion. In two spurs the placodal cells form a slender stream which distally is connected with the ganglion (Table 1).

8·0–9·2 mm. embryos, 22–23 days

In the 8·0 mm. embryo the IX and X nerves have grown considerably and are connected to the hind-brain by slender dorsal rootlets (Text-fig. 3). Proximally both nerves are commonly joined by persisting bridges of crest cells, but distally the petrosal and nodose ganglia are now independent. By the 9·0 mm. stage the

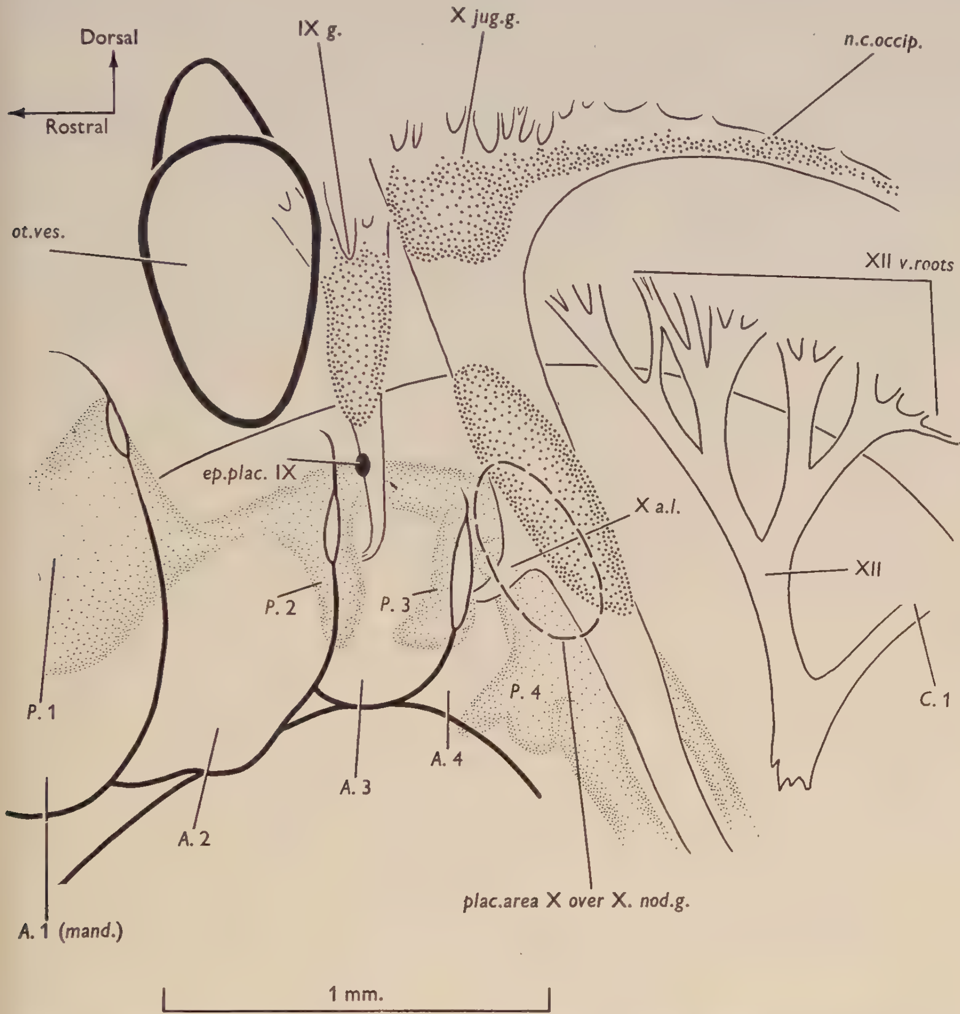


Text-fig. 2. Profile reconstruction of the left side of a 32-somite sheep embryo showing the relation of the glossopharyngeal and vagal neural crest to the epibranchial thickenings. Magnification ×50.

Table 1. *The number of placodal spurs, total number of migrating placodal cells and mitotic figures in the epibranchial placode of the vagus nerve in sheep embryos with* 26 *to* 38 *somites* (19–21 *days*). *In Tables* 1–4 *the number of connecting spurs is shown in brackets and the letter 'C' is used to denote embryos of Prof. Boyd's collection*

Embryo no.	Somite count	No. of placodal spurs		Total no. of placodal cells		Mitotic figures	
		Left	Right	Left	Right	Left	Right
10	26 s.	2	—	5	—	5	7
40	30 s.	1	2	4	7	7	6
28	32 s.	2	3	5	11	5	6
12	32 s.	—	—	—	—	8	6
13	32 s.	5	3 (1)	19	23	10	6
C 29	33 s.	—	2	—	6	5	7
11 A	33 s.	—	—	—	—	8	6
11 B	33 s.	1	—	2	—	12	10
14	35 s.	5	8 (1)	15	24	9	10
36	38 s.	4	3	16	14	7	6

proximal crest cells begin to concentrate against the lateral side of the vagus to produce a rudimentary jugular ganglion. Similar crest cells are encountered along the outer face of the spinal accessory nerve, which receives several minute fibrous roots from the medulla and from the cord as far caudally as the fifth cervical ganglion.



Text-fig. 3. Profile reconstruction of the left side of an 8·0 mm. sheep embryo. The placodal area of the vagus nerve now lies along the dorsal border of a shallow cervical sinus. Magnification × 50.

The nodose ganglion has grown into an elongated fusiform mass of young neuroblasts lying along the pharynx immediately dorsal to the third and fourth pouches. From its medial side a short branch passes into the mesenchyme of the fourth arch as the anterior laryngeal nerve. Distal to the nodose ganglion the visceral branch of the vagus courses along the side of the oesophagus to the level of the fourth cervical segment.

Epibranchial placode. At the 8 mm. stage the placode is still a flat thickening lying along the dorsal border of the shallow cervical sinus. During the growth changes which affect this region the ventral edge of the placode remains firmly attached to the closing plate of the third pouch, but the dorsal edge, being continuous with the ectoderm of the side of the head, becomes progressively elevated as the underlying mesenchyme grows in thickness. As a result of this differential growth the placode becomes tilted to face ventro-laterally in the 9 mm. embryo and the dorsal margin of the cervical sinus becomes more prominent.

Table 2. *The number of placodal spurs, total number of migrating placodal cells and mitotic figures in the epibranchial placode of the vagus nerve in* 8·0–9·2 *mm. sheep embryos* (22–23 *days*)

Embryo no.	Crown–rump length (mm.)	No. of placodal spurs		Total no. of placodal cells		Mitotic figures	
		Left	Right	Left	Right	Left	Right
C 8	8·0	3	4	12	20	20	18
15	8·0	5 (1)	6 (1)	36	24	27	17
4	8·0	7 (2)	5	34	22	12	26
561	8·0	3	5	13	22	9	20
16	8·2	2 (1)	7 (1)	15	53	29	41
17	8·2	2 (1)	—	12	—	27	18
43	8·2	9	3	34	9	40	41
C 9	8·5	2	4 (1)	9	18	18	13
18	8·5	2	6	8	26	19	15
19	8·5	7	7 (3)	35	42	42	21
42	8·7	6 (1)	8	41	33	33	37
C 13	8·7	4 (1)	2	15	13	43	31
C 14	9·0	1	—	4	—	24	31
C 3	9·0	2	3	5	15	10	13
20	9·0	3 (1)	6 (2)	19	37	29	20
25	9·0	9 (1)	9 (2)	61	46	31	37
21	9·0	8 (2)	1	51	6	35	31
537	9·0	7 (3)	10 (1)	37	54	33	48
552	9·0	11 (1)	3 (1)	48	23	33	19
8	9·0	5 (2)	2 (2)	118	40	12	8
48	9·0	9 (3)	7 (3)	79	55	32	29
49	9·0	5 (2)	2	29	9	20	26
551	9·2	—	1	—	3	19	16
C 20	9·2	4	8 (1)	17	44	27	29
C 21	9·2	4 (1)	5	18	40	19	26
550	9·2	7	11 (1)	69	129	16	22

The counts of dividing cells within the placode now range from nine to forty-eight, which indicates a marked rise in mitotic activity in comparison with the earlier embryos (Table 2). The wide individual variation in these counts further raises the possibility that the placode may show waves of proliferative activity. There is a widespread increase in the incidence of placodal spurs, one or more examples being present in forty-nine of the fifty-two placodes examined (94 % of the total). In 8 mm. embryos the total of placodal cells rarely exceeds forty, since the individual spurs usually contain fewer than six cells. But later, in the 9 mm. embryo, the formation of these spurs is more rapid and as some of them contain up to twenty cells the total is correspondingly higher.

The most striking evidence of the migration of cells from the placode lies in the presence of small continuous streams which extend from the placode to fuse intimately with the ganglion. A particularly clear connecting spur in an 8 mm. embryo is reproduced in Pl. 1, fig. 1*a*; this strand contains eight cells which were fortuitously

fixed during their migration through the mesenchyme to the nodose ganglion. Some of the smaller spurs appear less convincing on first examination, especially when they are cut obliquely or divided between two sections (Pl. 1, fig. 2*b*), but a careful scrutiny of serial sections is usually sufficient to establish their true form. Where this is uncertain the spur has not been recorded in the tables. The middle spur in the same section (Pl. 1, fig. 2*c*) contains three nuclei which have broken through the basement membrane and are about to migrate from the placode. The other two irregularities (Pl. 1, fig. 2*d*, *e*) are incipient papillae which are insufficiently developed to merit inclusion in the counts presented in Table 2. Occasionally the outline of the ganglion itself may bear an irregular projection of placodal cells which have already lost their connexion with the epithelium (Pl. 1, fig. 3*f*). But as so few of these ganglionic spurs are found it is reasonable to speculate that a migrating stream becomes quickly incorporated into the ganglion once the last cells have detached from the placode. The typical ganglionic spur illustrated in Pl. 1, fig. 3 consists of eleven cells together with a further five in the next section. The smaller migration point (Pl. 1, fig. 3*g*) has been obliquely cut, but the nucleus of a single migrating cell is just discernible.

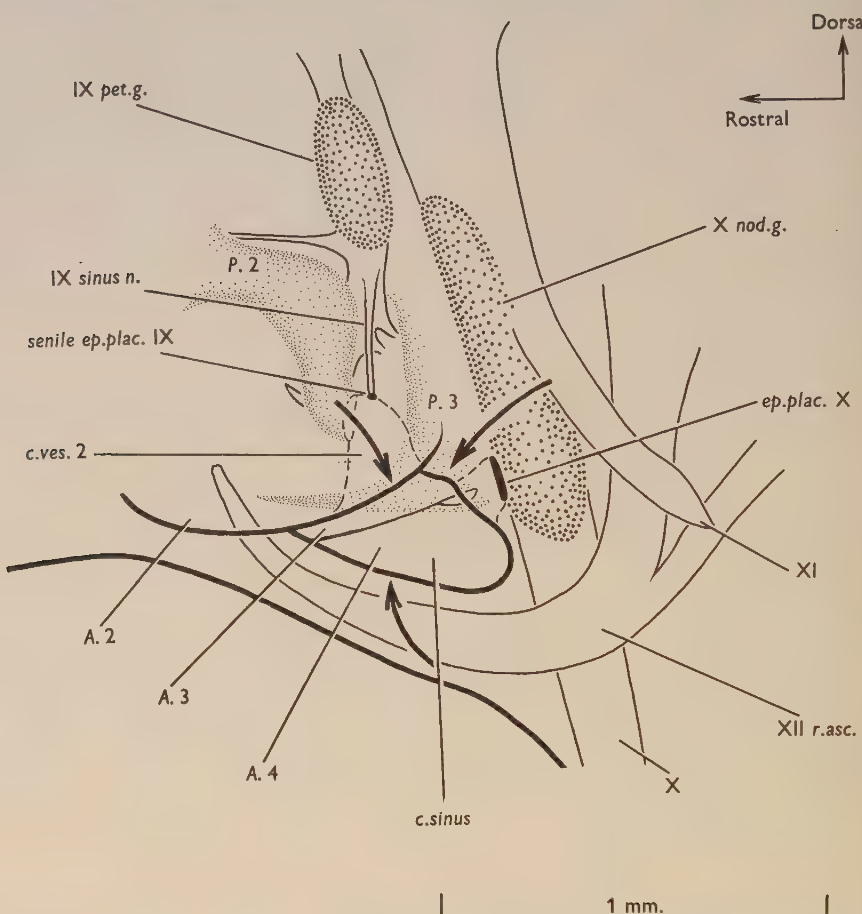
In 9·0 mm. embryos, placodal spurs are more regularly present and also more numerous in the individual placodes; seven of the placodes listed in Table 2 bear nine or more spurs. These findings point to a steady increase in the amount of cellular proliferation. Although small spurs are still found in every placode there is a trend towards the development of larger sites of detachment, especially in the later 9·0 mm. embryos. Three of these spurs contained thirty-eight, forty and sixty cells, respectively, and a typical example of a connecting spur with twenty cells is shown in Pl. 1, fig. 4*h*.

An additional feature brought out in Table 2 is the increase in the number of connecting spurs which are found both in the individual placodes and in the whole series. These spurs are present in seventeen of twenty-eight placodes (60 %) in 9 mm. embryos in comparison with ten out of twenty-four (41 %) in 8 mm. embryos. These values show that the increase in proliferation within the placode, as expressed by the count of mitotic figures and placodal spurs, is paralleled by an increase in cellular detachment and migration, as indicated by the numbers of connecting spurs.

9·5–10·5 *mm. embryos, 23–24 days*

The relationship of the epibranchial placode to the cervical sinus. During this period of development the cervical sinus becomes transformed into a deeper pit by the exuberant growth of the tissues which border it. The topographic changes which lead to the obliteration of the sinus as a surface feature are brought about by three different growth movements (Text-fig. 4, arrows). Along the cranial border the sinus becomes overlooked by the rapidly expanding second arch which encroaches upon, and then fuses with, the cranial face of the third arch, initially at a dorsal and slightly later at a ventral level. As a result, the closing plate of the second pouch and the adjacent epibranchial placode of the IX nerve become covered by a roof of second arch tissue, but still communicate with the diminishing caudal part of the sinus by a narrow flattened cleft, the cervical vesicle 2 (Garrett, 1948) or IX cyst (Frazer, 1925).

At the ventral border of the sinus the epipericardial ridge becomes a more prominent feature which provides a pathway for the entry of the ramus ascendens XII, and later for the migration of occipital myotomic material to the tongue rudiment. With the continued elevation of the dorsal border the epibranchial placode becomes further tilted and re-orientated to face ventrally or towards the



Text-fig. 4. Profile reconstruction of the cervical sinus region of the left side of a 10·5 mm. sheep embryo. The epibranchial placode of the vagus lies at the end of a short tunnel which opens into the deep cervical sinus. The arrows show the direction of the growth movements which lead to the closure of the cervical sinus. The anterior laryngeal nerve and the fourth pouch are not shown. Magnification ×50.

floor of the sinus. After the 10·5 mm. stage this dorsal edge begins to overhang the sinus so that the placode becomes hidden from view at the end of a short placodal tunnel. In the reconstruction the placode is now seen in profile (Text-fig. 4).

Epibranchial placode. The counts of dividing nuclei in the placode of 9·5–10·5 mm. embryos vary from nine to forty-three, which approximates to the range for the 8·0–9·2 mm. group and indicates that the level of mitotic activity is maintained (Table 3). In the 10·5 mm. embryos, however, the shorter range (ten to twenty-

seven) may suggest a gentle decline in the production of new cells in the placode. In the group as a whole, placodal spurs are found in thirty-four of the thirty-six placodes available, this being the same frequency (94 %) as in the 8·0–9·2 mm. group. In two cases where spurs are absent the placode is presumably in a state of temporary inactivity. The number of spurs developed in a single placode varies as widely as in the previous stage but, since the spurs also differ greatly in size, the total number of placodal cells provides a more reliable index of activity. While slender spurs of about ten cells are still commonly found, both in their resting and connecting form, the trend towards the development of much larger migration points becomes prevalent. The fact that some of these are massive streams containing between 100 and 250 cells helps to account for the high total figure. A comparison of Tables 3 and 4 confirms the impression that the migration of placodal cells is becoming more active especially in 10·5 mm. embryos. Connecting spurs are now present in 88·5 % of placodes in contrast to 60 % in 9·0 mm. embryos.

Table 3. *The number of placodal spurs, total number of migrating placodal cells and mitotic figures in the epibranchial placode of the vagus nerve in* 9·5–10·5 *mm. sheep embryos* (23–24 *days*)

Embryo no.	Crown–rump length (mm.)	No. of placodal spurs		Total no. of placodal cells		Mitotic figures	
		Left	Right	Left	Right	Left	Right
22	9·5	8 (2)	11 (3)	62	104	30	35
23	9·5	13 (9)	13 (5)	101	88	43	40
C X	10·0	3 (2)	3 (2)	115	36	9	19
24	10·0	6 (2)	8 (1)	63	39	36	42
29	10·0	2 (1)	—	13	—	33	26
542	10·0	7 (1)	6 (1)	42	32	25	24
536	10·0	10 (2)	6 (3)	92	77	17	17
547	10·0	2 (1)	4 (1)	13	34	20	10
548	10·0	3	2	23	23	14	24
540	10·5	9 (4)	4 (1)	265	89	13	20
544	10·5	—	4	—	40	22	26
413	10·5	10 (3)	6 (1)	142	73	26	24
539	10·5	7 (2)	7 (2)	230	150	17	19
562	10·5	6 (1)	11 (1)	324	134	14	11
553	10·5	5 (3)	4 (3)	287+	140	10	14
41	10·5	3 (1)	4 (2)	170+	170+	19	11
35	10·5	8 (3)	4 (3)	420+	420+	21	27
34	10·5	8 (3)	6 (2)	240+	175+	18	22

The histological appearance of the caudal region of the nodose ganglion at the 10·5 mm. stage is dominated by two features: the extremely intimate contact of the placode with the ventral side of the ganglion; and the mass migration of small cells from a wide area of the adjacent face of the tilted placode (Pl. 2, fig. 5). The overfolded shape of this particular placode is in marked contrast to the flat section displayed by earlier stages (cf. Pl. 1, figs. 1, 4). With the growth of the tissues at the dorsal border of the sinus the dorsal part of the placode has been carried ventrally through an arc of 130° and folded to face the original ventral part. Along the line of flexion the placode appears to lie in close contact with the ganglion, since the intervening film of mesenchyme, which was an obvious feature of the earlier stages (cf. Pl. 1, figs. 1–4), is now masked by the presence of numerous small placodal cells received during earlier phases of proliferation. The wedge of mesenchyme in the

angle between the deflected dorsal part of the placode and the ventro-lateral side of the ganglion is now occupied by a dense mass of small cells which spring from the placode by three separate spurs. The field shown in Pl. 2, fig. 5 contains over sixty cells, but the total for the whole connecting spur is more than 120 cells as it has a depth of 30 μ. These counts of placodal cells are conservative estimates as it is no longer possible to determine accurately the ventral edge of the ganglion.

An excellent example of another massive connecting spur is depicted in Pl. 2, fig. 6. Here the placode is not so sharply folded, but over a broad front placodal cells appear to be pouring out in a massive stream which is so intimately fused with the ganglion that the ventral edge of the latter is obscured. In this field about sixty placodal cells were counted, but the whole migration point has a depth of 50 μ in serial sections and contains more than 290 cells. In both Pl. 2, fig. 5 and Pl. 2, fig. 6, the dorsal zone of the ganglion is distinguished by the presence of young neuroblasts which have differentiated from neural crest cells.

11·0–12·7 *mm. embryos,* 25–27 *days*

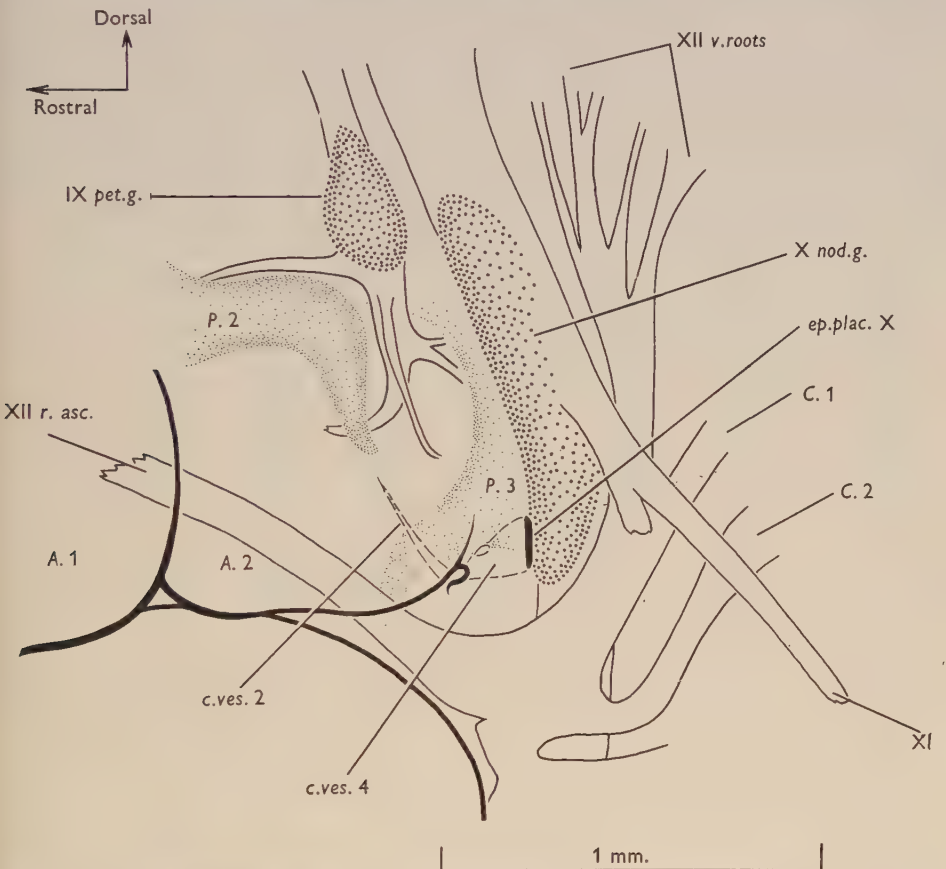
By the 11·5 mm. stage the open mouth of the cervical sinus has been constricted to a tiny pore which leads below the surface into two small chambers lined by ectoderm: the narrow tubular cervical vesicle 2 and the pyriform cervical vesicle 4 (Text-fig. 5). The cervical vesicle 4 represents a persisting relic of the caudal region of the original sinus since its floor contains minute areas of the third and fourth arches together with the intervening closing plate. The roof and blind inner end of the vesicle are formed by the folded epibranchial placode which is anchored to the nodose ganglion by massive streams of migrating cells.

Epibranchial placode. During this stage the rate of cell division within the placode appears to be of the same order as in the preceding stage, but the formation of placodal spurs is perhaps more rapid since one or more are regularly present in each of the forty-one placodes examined (Table 4). The universal presence of at least one connecting spur denotes a further increase in the rate of cell migration.

As in each of the earlier stages, there is some individual variation in the incidence of placodal spurs, and hence in the total number of cells which are about to migrate or actually are migrating. This is only to be expected if placodal activity is a repeating pattern in which the gradual formation of cellular spurs culminates in their detachment and rapid migration towards the ganglion. The total number of migrating cells in a placode thus reflects the balance at any moment between two interdependent activities: the formation of spurs, and their detachment. The two 25-day embryos, C35 and C36, appear to have a particularly low count of placodal cells, but this is due to the presence in each of a limited number of quite small spurs. Most other placodes at this stage contain several massive migration points so that the total is significantly higher. In another 25-day embryo, C37, the right placode has more spurs than the left, but since these individually contain fewer cells the total is much lower than on the left side. With the exception of these three comparatively inactive placodes it is clear from Table 4 that cellular migration takes place on a vigorous scale in the present stage. The values for the total number of cells refer only to those found within resting and connecting spurs, and thus express the present state of migration. But many small groups of placodal cells derived

from earlier phases of activity are found littering the ventral edge of the ganglion and even intruding into it. For this reason it is impracticable to attempt to determine the total number of placodal cells which the ganglion has already received, or is about to receive, from existing spurs.

Most 11–12 mm. embryos show transverse sections little different from that in Pl. 2, fig. 5, except that the placode is now buried in the roof of the cervical vesicle 4



Text-fig. 5. Profile reconstruction of the left side of an 11·5 mm. sheep embryo showing the cervical vesicle 2 and the cervical vesicle 4 still in communication with the surface by a narrow pore. The cervical vesicle 4 includes the closing plate of the third pouch, small areas of the third and fourth arches, and the epibranchial placode of the vagus. The anterior laryngeal nerve and fourth pouch are not shown. Magnification ×50.

and communicates with the exterior by a narrow pore at the extreme caudal end. This becomes occluded by the 12·5 mm. stage. As in the 10·5 mm. embryo the deflected part of the placode, which forms the roof of the vesicle, is the scene of a most active cellular migration which converts the intervening wedge of mesenchyme into a tightly packed fillet of placodal cells.

In the majority of embryos of this stage, and to a lesser degree in the preceding stage, occasional spurs of placodal cells proliferate to the adjacent face of the

anterior laryngeal nerve from the medial wall of the vesicle, which represents the originally ventral part of the early flat placode. These spurs are usually of fifteen to twenty-five cells, but larger examples of up to sixty cells are occasionally present.

13–16 *mm. embryos*, 27–28 *days*

Epibranchial placode. After the 13 mm. stage there is a steady fall in the rate of mitotic activity and little evidence of any further migration from the placode (Table 5). Pl. 2, fig. 6 illustrates the typical appearance of the nodose ganglion in a 13 mm. embryo at a level corresponding to that of Pl. 2, fig. 5. Here the fillet of small placodal cells is now clearly a part of the ganglion and the basement membrane of the placodal epithelium is partially restored. The altered shape of the ganglion contrasts strongly with its round cross-section at more proximal levels. The cervical vesicle 4 is still attached to the growing epidermis by a solid strand in 13 mm. embryos and the tension exerted by this transient anchorage explains the narrow compressed shape of the vesicle in transverse section. When this strand breaks at the 14 mm. stage the vesicle assumes a globular shape, but remains buried against the ventral side of the ganglion at least until the 22 mm. stage. In 16 mm. embryos the placodal fillet forms a characteristic feature of the ventro-lateral edge of the nodose ganglion for a distance of at least 80 μ cranial to the shrunken cervical vesicle (Pl. 2, fig. 8). The majority of cells within the fillet are now young neuroblasts which contrast strongly with the larger neuroblasts of crest origin in the dorsal sector of the ganglion.

Table 4. *The number of placodal spurs, total number of migrating placodal cells and mitotic figures in the epibranchial placode of the vagus nerve in* 11·0–12·7 *mm. sheep embryos* (25–27 *days*)

Embryo no.	Crown–rump length (mm.)	No. of placodal spurs		Total no. of placodal cells		Mitotic figures	
		Left	Right	Left	Right	Left	Right
C 36	11·0	1 (1)	2 (2)	45	60	9	19
C 35	11·0	2 (2)	1 (1)	40	20	22	20
30	11·0	4 (2)	3 (1)	270+	230+	21	23
538	11·0	4 (2)	1 (1)	150+	190+	16	15
545	11·0	5 (2)	lost	335+	lost	20	lost
C 37	11·2	5 (2)	6 (1)	210+	90	21	20
563	11·5	12 (5)	8 (4)	460+	340+	14	17
27	11·5	3 (3)	4 (3)	145+	135+	19	11
46	11·5	7 (2)	6 (1)	340+	250+	32	26
31	12·0	2 (1)	6 (4)	210+	270+	13	11
51	12·0	7 (3)	6 (1)	447+	265+	23	26
555	12·0	5 (4)	5 (2)	280+	180+	15	18
557	12·0	5 (3)	2 (1)	470+	470+	15	13
44	12·2	12 (3)	7 (3)	370+	260+	16	11
45	12·2	8 (5)	4 (2)	380+	318+	17	15
565	12·5	4 (2)	6 (2)	280+	360+	14	12
C 16	12·5	7 (4)	3 (2)	390+	220+	15	15
C 15	12·7	7 (3)	5 (4)	195+	185+	7	12
32	12·7	3 (3)	1 (1)	520+	320+	12	11
47	12·7	7 (2)	6 (2)	520+	645+	23	23
50	12·7	8 (2)	8 (2)	606+	735+	28	22

DISCUSSION

In the sheep the epibranchial placode of the vagus nerve develops from a broad area of thickened ectoderm which, as Halley (1955) has suggested in the cat, probably represents the fused placodes of the fourth, fifth and sixth arches. The appearance of the earliest placodal spurs coincides with the arrival of the nodose crest beneath this thickened ectoderm, but the crest does not fuse with the placode as in the cat (Halley, 1955). At least until the 10 mm. stage the two territories remain separated by a layer of mesenchyme in which migrating placodal spurs are easily distinguished.

The main changes in the pattern of migration and in the rate of cell division are summarized in Table 5. It is evident that placodal spurs are present in 65% of placodes in the first stage, in 94% in the 8·0–10·5 mm. stage, and thereafter in every example (100%) until migration ends rather abruptly about the 13 mm. stage.

Table 5. *Summary of cellular migration and mitotic activity in the epibranchial placode of the vagus nerve in 80 sheep embryos*

Stage	No. of placodes	No. of active placodes	Migrating placodal cells		Mitotic figures	
			Range	Mean	Range	Mean
26–38 somites	20	13	0–24	7·5	5–12	7·3
8·0–9·2 mm.	52	49	0–129	30·8	8–48	25·2
9·5–10·5 mm.	36	34	0–420	120·8	9–43	22·1
11·0–11·5 mm.	17	17	20–460	194·7	9–32	19·1
12·0–12·7 mm.	24	24	145–735	370·6	7–28	16·1
13·0–14·0 mm.	12	3	0–50	9·3	5–15	8·0

This suggests that the formation of spurs is an almost continuous process involving only short rest periods up to the 11 mm. stage. If this interpretation is correct, the pattern of activity of the vagal placode is strikingly different from that of the facial placode in the rat (Landacre, 1932) and the facial and glossopharyngeal placodes in the sheep (Batten, unpublished observations), all of which show irregular and intermittent activity.

As in a previous study of the trigeminal placode in the sheep (Batten, 1957), the presence of connecting streams has been accepted as evidence of a direct contribution to the ganglion. Only one author (Adelmann, 1925) seriously considered that the ganglion contributes cells to the placode and Landacre (1932) was able to refute this idea. The histological evidence for the migration of cells to the nodose ganglion is conclusive in itself, but is supported by the correlation which exists between the rate of mitotic activity in the placodal epithelium and the total number of cells migrating from the placode. The peak of mitotic activity apparently precedes the phase of most intense migration and then declines to a low value for the senile placode of the 13 mm. stage (Table 5).

The marked individual variation in the incidence of placodal spurs, and hence in the total number of migrating cells, is a feature common to the placode at all stages. A partial explanation of this lies in the fact that the actual migration of cells probably occurs intermittently. In any event the number of spurs reflects the state of the placode at only one particular moment during a period of activity which lasts for 9 days. It is worth recalling Landacre's argument that the absence of evidence

of proliferation in one, or even several, placodes in mammalian embryos does not preclude the possibility of migration occurring (Landacre, 1932). For these reasons, as well as the positive evidence in other embryos, it is justifiable to accept as temporarily inactive those placodes which show no spurs or migrating cells.

The history of the epibranchial placode in the sheep falls into two periods which are distinguished by differences in the intensity of migration and in the relation of the placode to the ganglion. During the early period, which lasts until the 9 mm. stage, the placode lies flat upon the surface of the embryo and detaches cells either singly or in small spurs of up to twenty-five cells. Although it is not possible to determine the fate of these cells after they enter the ganglion there is no evidence to suggest that they degenerate, and it is probable that they may differentiate into neuroblasts or sheath cells or even both. Campenhout (1936, 1937, 1948) has described similar budding in the pig, chick and human and has assumed that the detached placodal cells become neuroblasts. Coërs (1946) mentions an epibranchial contribution to the nodose ganglion in the rabbit.

During the later period of activity the placode becomes involved in the topographic changes which lead to the disappearance of the cervical sinus. This phase is characterized by the formation of massive placodal spurs and intense migration, the total contribution far exceeding that provided by the facial and glossopharyngeal placodes. The detailed account of the closure of the cervical sinus confirms the description of Scothorne (1950), and indicates that the surface changes are fundamentally similar to those in the human (Frazer, 1925; Garrett, 1948) and the cat (Halley, 1955). The anchorage of the cervical vesicle 4 to the nodose ganglion must be correlated with the fact that the placode is engaged in contributing cells to the ganglion during the final stages of closure of the sinus. As in the earlier stage a limited number of placodal cells invade the ganglion, but when migration becomes more vigorous the placodal cells begin to accumulate in the intervening wedge of mesenchyme. By the 12 mm. stage this region has been converted into a tightly packed mass of placodal cells, among which young neuroblasts begin to appear at the 13 mm. stage. Later this placodal fillet becomes an integral part of the ganglion and by the 16 mm. stage the few remaining placodal cells are outnumbered by neuroblasts of several sizes. The appearance of neuroblasts in a cellular mass which is exclusively placodal in origin, together with the fact that the young neuroblasts increase in number at the expense of placodal cells, offers evidence of the transformation of placodal cells into neuroblasts. The nodose ganglion in the sheep thus has a dual origin; the greater part is formed from the neural crest and a small part at the caudal pole is provided by the epibranchial placode.

While it is obvious that no firm statement can be made on the total number of cells received by the nodose ganglion throughout the period of placodal activity, it might be of interest to speculate on this point. The mean values for migrating placodal cells shown in Table 5 give some indication of the changing intensity of migration at the different stages, but it would be misleading to accept their sum—733 cells—since these mean values minimize the extreme individual variation which is produced by a pattern of recurring formation and detachment of placodal spurs. On the assumption that the total accession is constant in all embryos, it might be preferable to compute a total by combining the numbers of migrating cells found in

the most active placode at each of the stages used in the description. Allowing a sufficient interval between the selected placodes, in order to minimize the possibility of duplicating a massive spur which would presumably be slow in detaching, this total is estimated to be of the order of 1300 cells (Table 6). Of this total, 176 cells become incorporated in the ganglion during the early stage of proliferation and the remainder become packed together outside the ganglion as the placodal fillet.

The full duration of placodal activity has been appreciated only by those workers who have traced the inclusion of the placode within the cervical vesicle 4. In citing the 8 mm. stage as the end of activity in the pig, Campenhout (1936, 1937) overlooked the phase of maximum intensity, which Winiwarter (1938, 1939) placed at the 10 mm. stage. Halley (1955) has pointed out that the nodose ganglion in the cat continues to receive cells from the placode when it becomes submerged in the cervical vesicle at the 10 mm. stage. At the same stage in the rabbit Coërs (1946) found a bridge of small cells between the placode and the ganglion, but was uncertain of their placodal origin. There is little doubt, however, that Völker (1922) was the first to observe the fate of the placodal cells which are added to the nodose

Table 6. *A tentative estimate of the total number of placodal cells received by the nodose ganglion during the activity of the epibranchial placode*

Stage	No. of migrating cells in the most active placode	
35-somites	24	=176 cells incorporated in the ganglion
8·0 mm.	34	
9·0 mm.	118	
10·5 mm.	420	=1155 cells in the placodal fillet
12·7 mm.	735	
Total	1331 placodal cells	

ganglion in a mammal. He described in the squirrel the formation of a cellular mass which corresponds exactly with the placodal fillet in the sheep and found that these cells were entirely replaced by neuroblasts in the 20 mm. embryo.

A suggestion that the visceral neurons in the VII, IX, and X ganglia in all vertebrates are derived from the endoderm was originally put forward by Dart & Shellshear (1921), but this idea has not been generally accepted. Although Coërs (1946) described some meagre endodermal contribution to the geniculate and petrosal ganglia in the rabbit, he denied the existence of an endodermal placode in relation to the nodose ganglion. Other authors have made no reference to endodermal proliferation either in mammals (Campenhout, 1936, 1937; Halley, 1955; Völker, 1922) or in fish and amphibia (Landacre, 1908–33, and others). In the sheep, too, there is no conclusive evidence of an endodermal contribution to the nodose ganglion at any stage up to the 22 mm. embryo. The outstanding exception is Winiwarter (1938, 1939), who found endodermal migration occurring all over the derivatives of the third pouch in 15 mm. pig embryos and ending with ‘une véritable fonte des cellules épithéliales dans le massif nerveux’ (Winiwarter, 1938, p. 1192). This description is strongly reminiscent of the vigorous cellular migration which the X placode shows when it becomes submerged in the cervical vesicle 4 in the cat, squirrel and sheep. Moreover, this vesicle includes in its wall the closing plate of

the third pouch, so that it could easily be mistaken for an endodermal structure, as Winiwarter appears to have done, especially if the transient connexion with the ectoderm is overlooked.

The present investigation has shown that there is reliable evidence of the differentiation of epibranchial placodal cells into neuroblasts in the sheep and also in the squirrel (Völker, 1922), but their significance remains uncertain. From evidence in certain fishes, where the taste-bud system is greatly hypertrophied, Landacre (1908, 1910) has concluded that the epibranchial placodes provide the special visceral efferent component. He expressed the same opinion in a study of the facial placode in the rat (Landacre, 1932). Coërs (1946), however, states that gustatory fibres are absent in the vagus nerve in the rabbit and supposes that the placode might provide general cutaneous fibres. Most textbooks of human neuro-anatomy agree that the vagus nerve contains a limited number of taste fibres, with cell bodies in the nodose ganglion and peripheral fibres coming from taste buds in the epiglottis (Wilson, 1905; Feindel, 1957). Although there appear to be no references to the components of the vagus in the adult sheep it is tempting to assume from the conditions in the lower forms that the placodal neuroblasts may relate to gustatory sensation. This view would be consistent with the concept of the functional specificity of epibranchial placodes which Ariëns Kappers (1941) considers valid for all classes of vertebrates.

SUMMARY

1. The epibranchial placode of the vagus nerve at first lies along the dorsal border of the cervical sinus, but later becomes incorporated in the inner wall of the blind cervical vesicle 4.

2. From the nineteenth to the twenty-seventh day the placode is actively engaged in proliferating cells to the neural crest rudiment of the nodose ganglion. The earliest spurs appear about the 26–32-somite stage as small papillae of up to four cells.

3. About the 8 mm. stage a larger type of placodal spur with up to thirty cells becomes more frequent and the total number of migrating cells varies between 20 and 100.

4. The presence of streams of placodal cells connected with the ganglion is accepted as evidence of a cellular contribution. The wide individual variation in the incidence of connecting spurs suggests that detachment occurs intermittently, but the formation of new spurs is an almost continuous process.

5. Around the 10 mm. stage the placode enters a final phase of vigorous migration which involves massive spurs of up to 250 cells. Detached placodal cells accumulate to form a fillet which is interposed between the placodal roof of the cervical vesicle and the ganglion.

6. With the appearance of young neuroblasts among these placodal cells at the 13 mm. stage the fillet becomes an integral part of the ganglion. In 16 mm. embryos the majority of these placodal cells have differentiated into neuroblasts.

7. It is not possible to trace the fate of placodal cells which enter the ganglion during the early phase of activity up to the 10 mm. stage. In the absence of

evidence of their degeneration it is assumed that these cells take part in the formation of the ganglion, possibly by differentiating into sheath cells or neuroblasts.

8. The anterior laryngeal nerve receives at all stages a limited number of placodal cells, but their ultimate fate cannot be determined.

9. The total output during the period of active proliferation is estimated at about 1300 placodal cells.

LIST OF ABBREVIATIONS

*A.*1	pharyngeal arch 1
*A.*1 (*mand.*)	mandibular process
*A.*2	pharyngeal arch 2
*A.*3	pharyngeal arch 3
*A.*4	pharyngeal arch 4
c.sinus	cervical sinus
*c.ves.*2	cervical vesicle 2
*c.ves.*4	cervical vesicle 4
*C.*1	first cervical nerve
*C.*2	second cervical nerve
ep.plac. VII	facial epibranchial placode
ep.plac. IX	glossopharyngeal epibranchial placode
ep.plac. X	vagal epibranchial placode
plac.area X	approximate limits of vagal placode
Froriep's g.	Froriep's ganglion
mes.	mesencephalic flexure
n.c. V	trigeminal neural crest
n.c. VII	facial neural crest
n.c. IX–X	vago-glossopharyngeal neural crest
n.c.occip.	occipital neural crest
n.c.X jug.	neural crest rudiment of jugular ganglion
op.ves.	primary optic vesicle
ot.ves.	otic vesicle
*P.*1	pharyngeal pouch 1
*P.*2	pharyngeal pouch 2
*P.*3	pharyngeal pouch 3
*P.*4	pharyngeal pouch 4
IX	glossopharyngeal ganglion
IX *pet.g.*	petrosal ganglion
IX *sinus n.*	sinus nerve
X	vagus nerve
X *a.l.*	anterior laryngeal nerve
X *jug.g.*	jugular ganglion
X *nod.g.*	nodose ganglion
XI	spinal accessory nerve
XII *v.roots*	ventral roots of hypoglossal nerve
XII *r.asc.*	ramus ascendens of hypoglossal nerve
*som.*1	somite 1

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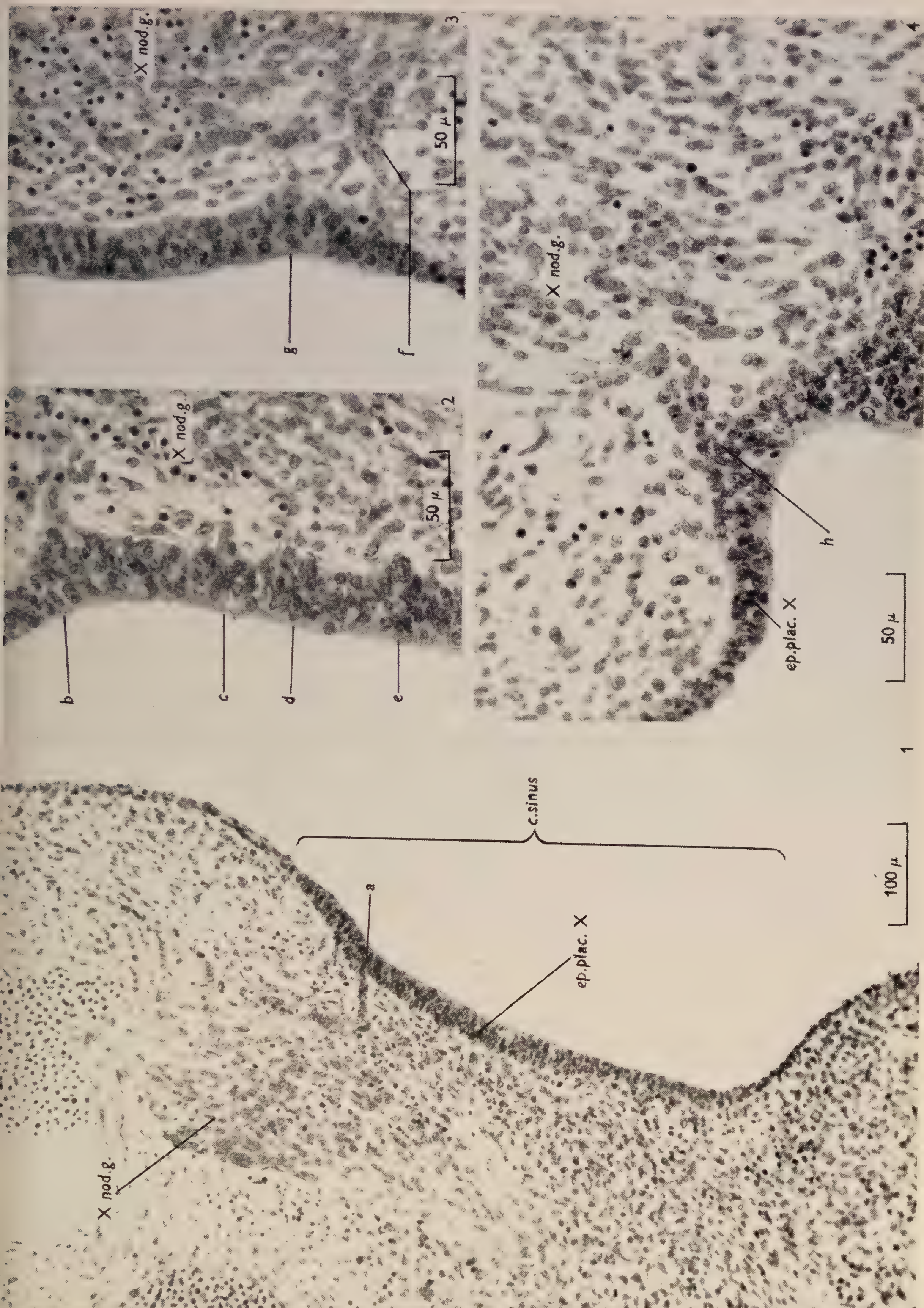
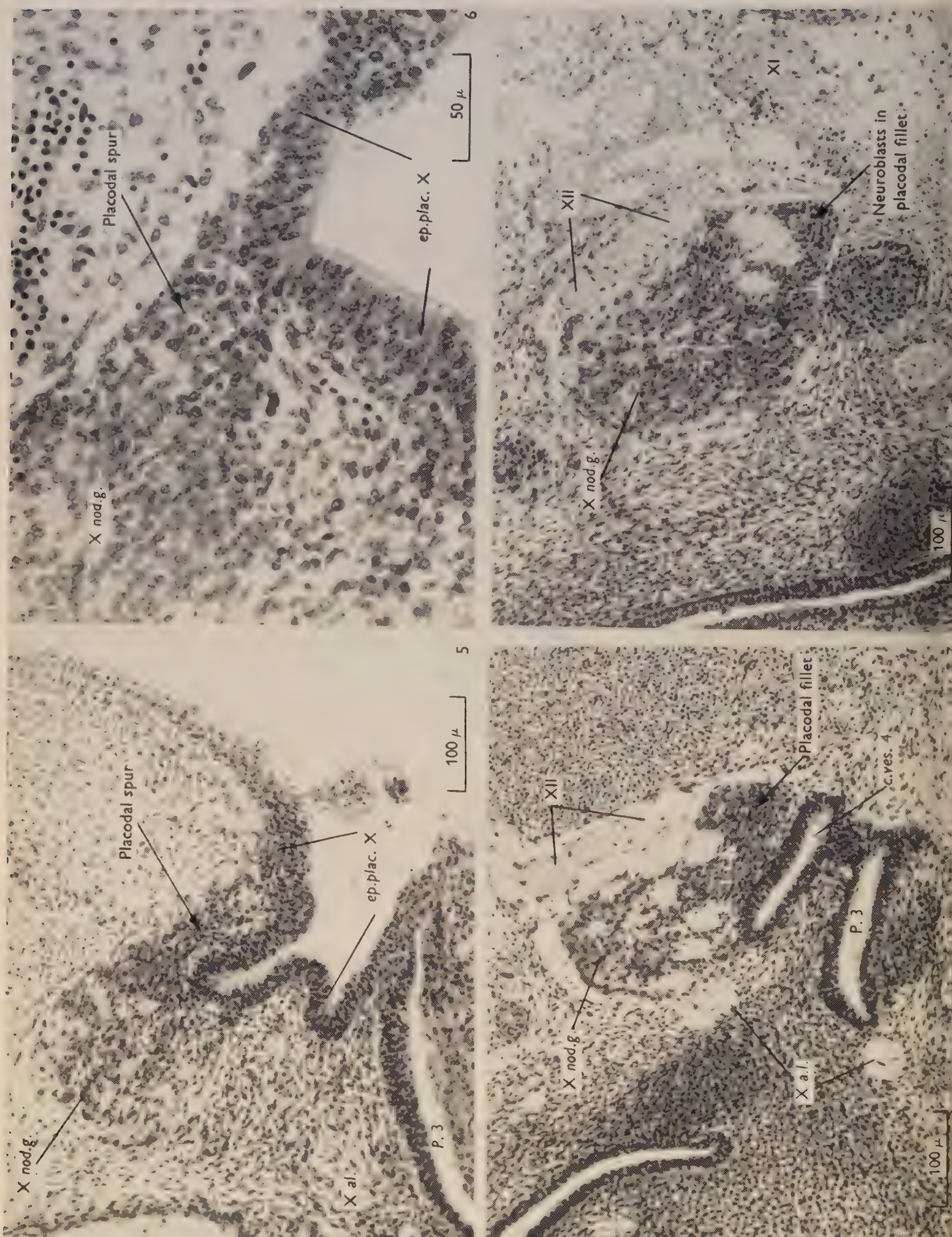


BATTEN—Epibranchial placode of the vagus nerve

(*Facing p.* 488)



BATTEN—Epibranchial placode of the vagus nerve

EXPLANATION OF PLATES

Plate 1

Fig. 1. Coronal section through the nodose ganglion, epibranchial placode and the dorsal part of the shallow cervical sinus in an 8·2 mm. sheep embryo. The connecting spur (*a*) contains eight cells.

Fig. 2. Coronal section from an 8·2 mm. embryo showing a small connecting spur (*b*) which has been divided between two sections, a small resting spur (*c*) containing three nuclei and two incipient papillae (*d* and *e*). Note the layer of mesenchyme between the placode and the ganglion.

Fig. 3. Coronal section from the same embryo as Fig. 2 showing a spur of placodal cells projecting from the ganglion (*f*) and a single nucleus leaving the placode (*g*).

Fig. 4. Transverse section from an 8·5 mm. embryo showing a connecting spur (*h*) of twenty cells, which is fused with the ganglion.

Plate 2

Fig. 5. Transverse section from a 10·5 mm. embryo showing an overfolded placode in close contact with the ganglion. The deflected dorsal part of the placode bears a massive connecting spur which is receiving cells from three separate sites. Note the neuroblasts of neural crest origin in the dorsal part of the ganglion.

Fig. 6. Transverse section from another 10·5 mm. embryo showing a massive connecting spur with over sixty cells. The whole spur has a depth of 30 μ and contains over 290 cells.

Fig. 7. Transverse section from a 13 mm. embryo at a level similar to that of Fig. 5. There is now no evidence of migration and the placodal cells are massed together as a fillet in which young neuroblasts are present.

Fig. 8. Transverse section from a 16 mm. embryo showing the nodose ganglion a short distance ahead of the cervical vesicle. Note the alteration in the shape of the ganglion due to the addition of placodal cells. The placodal edge of the ganglion consists of small neuroblasts, but elsewhere the ganglion contains larger neuroblasts which have differentiated from the neural crest.

THE DEVELOPMENT OF THE HUMAN VAGINA

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The literature on the development of the human vagina is already so abundant that this further contribution is made only with some trepidation. However, the findings which form the substance of this paper seem of sufficient significance to be recorded, since they clearly demonstrate a method of vaginal development which has not so far been generally accepted in this country. The material studied, while not always forming as complete a series as might be wished, has in most cases been in excellent condition for detailed histological examination, and it is possible that the previous confusions and disagreements on this subject were largely due to deficiencies in the material available to earlier workers.

Bloomfield & Frazer (1927) pointed out that the descriptions of vaginal development in the literature could be divided into three main groups, depending on the structures which their authors believed to take part in the formation of the vagina, and it will be convenient to employ this classification here.

In the first group are those accounts which have attributed the origin of the entire vagina to the lower end of the Müllerian utero-vaginal canal. This opinion first gained general acceptance from the demonstration by Thiersch and others, work which is well summarized by Banks (1864), of the fusion of the Müllerian ducts in the genital cord, and in this century has been supported by Felix (1912), Bloomfield & Frazer (1927), Hunter (1930) and von Lippmann (1939). It may be regarded as the 'classical' account of vaginal development, and was for many years the standard textbook description.

The second group consists of those descriptions which supposed that the vaginal epithelium was formed in part (Tourneux & Legay, 1884; Mijsberg, 1924) or in whole (Hart, 1896, 1901 and 1911; Kempermann, 1931) from the lower ends of the Wolffian ducts.

The third group includes the accounts of all those workers who believed that the epithelium of the urogenital sinus made a contribution to the vagina. Before the time of Thiersch it was accepted that the entire vagina was derived from the sinus (Müller, 1830; Rathke, 1832; Valentin, 1835), but this opinion was discarded when the fusion of the Müllerian ducts became known. Retterer (1891), however, suggested that while most of the vagina was formed from the utero-vaginal canal a short lower segment arose by the splitting of the upper part of the sinus into dorsal and ventral channels, and this view was later supported by Bolk (1907). Koff (1933), whose work is recognized by most current British and American textbooks, believed that the lower one-fifth of the vagina was derived from the sinus by the growth of paired epithelial 'sino-vaginal bulbs' from the dorsal sinus wall, these bulbs fusing together to form a lower vaginal segment. On the other hand, Spuler (1930), Vilas (1932), Kempermann (1935) and Meyer (1934–38)

believed that a proliferation of cells from the dorsal wall of the sinus gave rise to the epithelial lining of the entire vagina, displacing the epithelium of the utero-vaginal canal as far as the cervix. This opinion has recently been supported by Politzer (1955), and Zuckerman (1940) showed that there was a considerable body of endocrinological evidence in its favour.

MATERIAL

The material consists of thirteen embryos and foetuses, ranging from 28 to 375 mm. crown–rump length, and an infant of 1 month. The majority of the specimens were already sectioned and stained with haematoxylin and eosin, alternately with haematoxylin and eosin and a trichrome stain, or by the de Castro method of silver impregnation. The remainder were sectioned at 6–10 μ, either transversely or coronally, and the sections stained mainly with haematoxylin and eosin or a trichrome stain. With some of the larger specimens slides were also stained with Best's ammoniacal carmine and by the periodic acid-Schiff technique (Gomori, 1952).

28 *mm. embryo*

In the lower part of the genital cord the Müllerian ducts are in apposition with each other, their medial walls forming a septum between the two lumina. They are lined by a closely packed columnar epithelium and lie between the two Wolffian ducts, rather larger structures with a cubical epithelium. The caudal ends of the Müllerian ducts do not quite reach the dorsal wall of the sinus, remaining separated from the Müllerian tubercle by a small mass of mesoderm, but on either side they are in close contact with the lower ends of the Wolffian ducts (Pl. 1, fig. 1).

Most of the pars pelvina of the sinus is lined by an epithelium consisting of three or four layers of small, darkly staining cells with closely arranged nuclei, but in the region of the Müllerian tubercle the epithelium of the dorsal sinus wall is differentiated into a thin basal layer of deeply staining cells overlain by one to three layers of larger and very pale staining cells with relatively smaller nuclei. At the Wolffian openings, on either side of the Müllerian tubercle, these pale cells seem to be compressed together, and they extend backwards for a short distance into the lower ends of the Wolffian ducts themselves.

42 *mm. foetus*

The lower portions of the Müllerian ducts are completely fused together as the utero-vaginal canal and the caudal tip of the Müllerian epithelium forms a solid mass in contact with the dorsal wall of the sinus, though a small central mass of mesoderm interrupts this contact in the midline. On either side of the Müllerian tubercle the Wolffian ducts enter the sinus, and are now of about the same calibre as the utero-vaginal canal. The sinus epithelium presents the same features as in the 28 mm. specimen (Pl. 1, fig. 2).

50 *mm. foetus*

This specimen shows no significant developmental change, except that the sinus epithelium in the region of the Müllerian tubercle is now considerably thicker. This epithelium, divided into basal deeply staining and superficial pale-staining zones, will in future be referred to as the differentiated type of sinus epithelium.

65 *mm. foetus*

The Müllerian utero-vaginal canal is a large structure, oval in cross-section and with its long axis lying transversely (Pl. 1, fig. 3). Throughout most of its extent it is lined by a closely packed columnar epithelium, but about 0·3 mm. above the caudal end the lining cells lose their columnar arrangement and form a thick stratified polygonal epithelium, which at the lowermost tip completely occludes the lumen. The Wolffian ducts are very small structures, lined by a cubical epithelium, and lie on either side of the utero-vaginal canal. There is a short segment where the Wolffian ducts have completely disappeared, just above their lower ends, but they reappear below this to join the dorsal wall of the sinus.

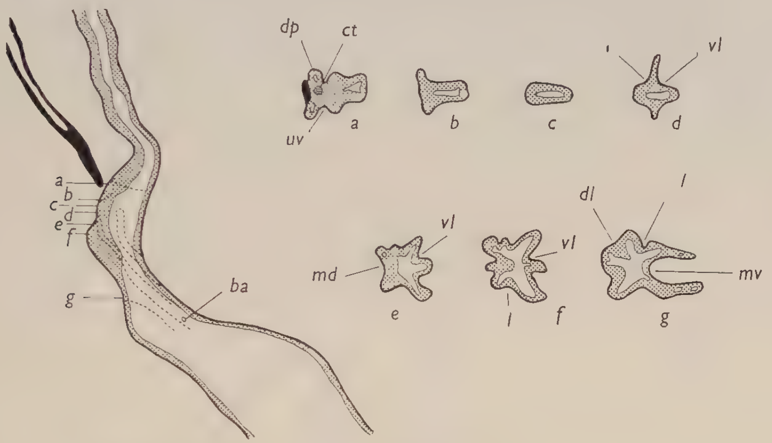
The urogenital sinus shows a very significant advance in the region previously occupied by the Müllerian tubercle (Pl. 1, figs. 4, 5, 6). On either side the Wolffian ducts join 'dorso-lateral projections' of the sinus, solid epithelial structures which prevent the communication of the ducts with the sinus lumen and are in contact on their dorso-medial aspects with the solid mass of Müllerian epithelium at the caudal end of the utero-vaginal canal. More medially, the Müllerian epithelium is displaced dorsally by a proliferation of darkly staining cells from the dorsal sinus wall, between the bases of the two dorso-lateral projections (Pl. 1, fig. 4). The dorso-lateral projections are each formed by a central mass of pale-staining cells, with small nuclei, surrounded by a thin basal layer of more darkly staining cells, and the cubical cell lining of the lower ends of the Wolffian ducts is applied to their dorsal aspects. Caudal and ventral to the proliferation of darkly staining cells from the dorsal sinus wall, the pale-staining cells extend forwards and medially to form a crest projecting into the dorsal aspect of the sinus lumen (Pl. 1, figs. 5, 6)—the structure identified by Kempermann (1931) as the *Wolffsche Kamm*. Apart from this differentiation in the region previously occupied by the Müllerian tubercle, the remainder of the pars pelvina is lined by a darkly staining stratified epithelium of six or seven layers of small cells.

In this specimen, therefore, the sinus cells in the region which was previously lined by the differentiated type of epithelium form a proliferative zone, apparently composed of three elements. On either side are the dorso-lateral projections, associated with the Wolffian openings, while between them is a proliferation of darkly staining cells from the dorsal sinus wall.

68 *mm. foetus*

This specimen is at only a slightly later developmental stage than the 65 mm. foetus, but several interesting differences may be noted. The Wolffian ducts are present throughout the whole of the genital cord as small, but easily recognizable structures. The occlusion of the lumen of the caudal portion of the utero-vaginal canal reaches further cranially than in the 65 mm. specimen, to a distance of about 0·3 mm. above the junction of Müllerian and sinus epithelia. A small mesodermal mass is enclosed in the root of the proliferation of darkly staining cells from the dorsal wall of the sinus, splitting it into bilateral elements which meet each other behind the mesodermal mass and are there in contact with the caudal end of the Müllerian epithelium (Text-fig. 1*a*).

The most interesting feature is the appearance of a characteristic system of longitudinal folds in the sinus wall (Text-fig. 1), foreshadowed at the 65 mm. stage but now very much more distinct. It may be noted that there is a pronounced fold—termed the 'urethro-vaginal fold'—demarcating from the rest of the sinus its cranial and dorsal portion which forms the zone of contact with the Müllerian tissue. The dorsal margins of the urethro-vaginal folds run from the dorsal wall of the sinus, below the dorso-lateral projections, to meet each other behind the lower end of the urethra, and limit the area, dorsal and cranial to them, which is occupied by the



Text-fig. 1. 68 mm. foetus. The figure on the left is a graphic reconstruction (× 20) of a median sagittal section through the lower end of the utero-vaginal canal and the urogenital sinus. The figures on the right, *a–g*, are transverse sections at the levels indicated in the sagittal section. The dorsal aspect of the sections is towards the left. The Müllerian epithelium is shown by the solid black shading, and the sinus epithelium by the stippling. A small area of connective tissue, *ct* in fig. 1*a*, interrupts the sinus proliferation in the midline. The folds of the sinus wall are indicated in the transverse sections, and their relative positions in the sagittal section are shown by the dotted lines. *dl*, dorso-lateral fold; *md*, median dorsal fold; *l*, lateral fold; *vl*, ventro-lateral fold; *uv*, urethro-lateral fold; *v*, median ventral fold. The dorso-lateral sinus bay lies between the lateral and dorso-lateral folds (figs. 1*f*, 1*g*) or between the lateral and median dorsal folds (fig. 1*e*), and is joined at *ba* by the duct of Bartholin's gland. The ventro-lateral bay is between the lateral and ventro-lateral folds. It will be noticed that the relief of the sinus wall compares very closely with that described by Mijsberg (1924) and Politzer (1952) at similar developmental stages.

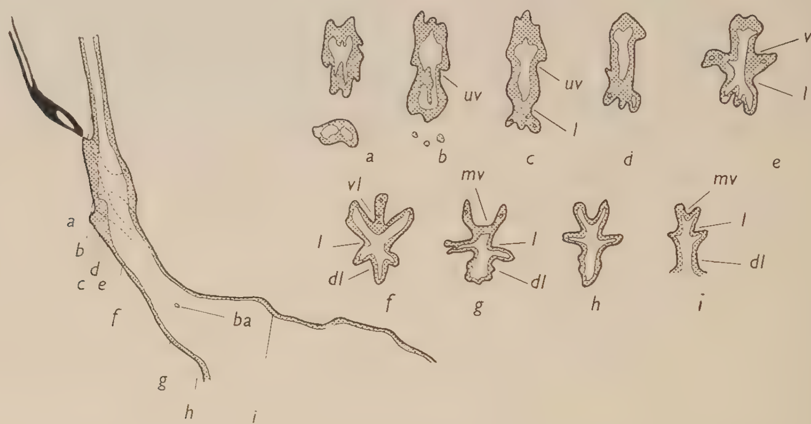
differentiated type of sinus epithelium. The pattern of the folds in the wall of the human sinus has been described previously by Mijsberg (1924) and Politzer (1952). They are indicated in the diagrams in Text-figs. 1–4, and a more detailed account has been given elsewhere (Bulmer, 1955).

14 *week foetus*

The dorso-lateral projections can be identified, similar in structure to those of the 65 mm. stage, and their dorso-medial aspects are in contact with the Müllerian epithelium. Between their bases is the mass of darkly staining cells proliferating from the dorsal wall of the sinus, ventral to the Müllerian epithelium and now more extensive than in the 65 mm. foetus.

94 *mm. foetus*

There is a considerable increase in size of the sinus compared with the 68 mm. stage (Text-fig. 2), but the arrangement of the longitudinal folds remains essentially unchanged. The urethro-vaginal fold is a marked feature, accentuated by the enlargement of the sinus which lies dorsal and cranial to it (Text-fig. 2*b*). This enlargement is associated with a considerable thickening of the epithelium, which now consists of a basal zone of five or six layers of small, darkly staining cells, with relatively large nuclei, clearly demarcated from a superficial zone of three or four layers of larger, clear staining polygonal cells with relatively much smaller nuclei. There is a striking distinction between the two zones in the low-power view (Pl. 2, fig. 7), and this epithelium is markedly different from the much thinner, undifferentiated type of epithelium, of four or five layers of smaller, rather darkly staining cells, which lines the rest of the pars pelvina.



Text-fig. 2. 94 mm. foetus. Median sagittal section through the sinus and the lower end of the vagina, with the corresponding coronal sections ($\times 13\frac{1}{2}$). Shading and lettering as for Text-fig. 1. The dotted lines in the sagittal section indicate the relative positions of the folds in the upper part of the sinus wall. The coronal sections, *a–i*, are shown with their cranial ends (i.e. the ventral wall of the sinus) towards the top.

The enlargement of the sinus behind the urethro-vaginal folds also forms the root of a short 'sinus upgrowth', projecting dorsally and cranially from the sinus to meet the caudal end of the Müllerian epithelium. The lower end of the sinus upgrowth contains paired lumina, continuous with the sinus lumen, and the epithelium is of the same differentiated type as that of the sinus enlargement. Followed further cranially the lumina disappear, and the sinus upgrowth forms a solid crescentic mass of epithelium in which the basal and superficial zones are still distinct (Pl. 2, fig. 8), though the basal cells are not so prominent as they are more caudally. In addition, a small mass of darkly staining cells, the caudal end of the Müllerian tissue, is embedded in this cranial end of the sinus upgrowth.

As the vaginal mass is followed still further cranially the left side of the sinus upgrowth is joined by a short persistent segment of the lower end of the left Wolffian duct, and the Müllerian epithelium comes to occupy a gradually increasing area in

the centre. Eventually, about 240 μ above the root of the sinus upgrowth, the sinus cells are completely replaced by the solid Müllerian epithelium, which is canalized a short distance above this as the lower end of the utero-vaginal canal. This part of the canal is lined by a three- or four-layered stratified polygonal epithelium, which extends cranially to meet the columnar cell lining of the upper part. The level of junction between these two types of Müllerian epithelium coincides with a fusiform swelling of the genital cord, corresponding with that identified by Koff (1933) as the site of the future cervix.

It can be appreciated that several changes have occurred to reach this stage of development. The three components which formed the proliferation of the sinus from the 65 mm. stage onwards—the two dorso-lateral projections and the darkly staining cells between them—have apparently fused together to form a single mass which extends dorsally and cranially as the sinus upgrowth. The lower ends of the Wolffian ducts have disappeared except for a short persistent segment on the left side, which, as might be expected, joins the side of the sinus upgrowth. In addition, the 'vaginal' portion of the utero-vaginal canal, so far as this can be defined, is now entirely lined by a stratified polygonal epithelium, presumably derived from the original columnar Müllerian epithelium. One of the most interesting features, however, is the differentiation of the sinus epithelium behind and above the urethro-vaginal folds. A similar differentiation, though less marked, has been noted since the 28 mm. stage, always confined to this particular region of the sinus, and it is from this differentiated type of epithelium that the sinus upgrowth appears to arise.

112 *mm. foetus*

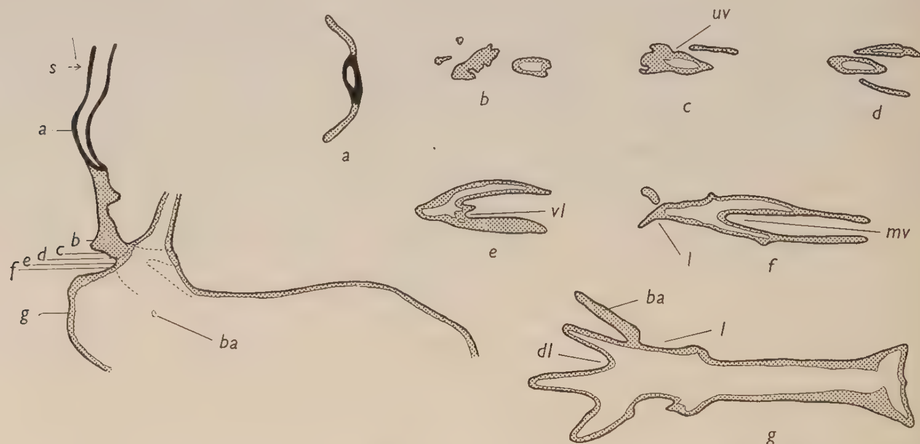
The configuration of the urogenital sinus shows little change from the 94 mm. stage, though the pars phallica is now becoming more dorso-ventrally elongated. The sinus upgrowth extends for about 570 μ above the dorsal wall of the sinus, as a transversely elongated epithelial plate, and its cranial end reaches up for a short distance as tapering 'wings' on either side of the lower end of the utero-vaginal canal. Caudally it is continuous with the dorsal wall of the sinus, but its root is split into two by a small mesodermal septum (Pl. 2, fig. 9). Dorsal to this septum the two roots join each other to form the sinus upgrowth, while on its ventral aspect they are continuous with the enlarged dorsal and cranial portion of the sinus which lies above and behind the urethro-vaginal folds. This arrangement obviously cannot have arisen from conditions such as have been described in the 94 mm. foetus. Apparently, as in the 68 mm. specimen, a small mesodermal septum must have divided the root of the sinus upgrowth at the time of its initial formation.

The epithelium of the sinus upgrowth stains rather more deeply than in the 94 mm. foetus, but the basal and superficial zones can be readily recognized and it can be distinguished from the Müllerian epithelium with which it is in contact (Pl. 2, fig. 10). At the caudal end of the upgrowth, and in the enlargement of the dorsal portion of the sinus from which it arises, the epithelium is of the same differentiated type, but here the pale-staining cells are much larger and more prominent than they are further cranially, their cytoplasm eosinophilic and their nuclei very small (Pl. 2, fig. 9). The remainder of the pars pelvina is still lined by the same undifferentiated type of epithelium as in the earlier stages.

The lower part of the utero-vaginal canal is lined by a stratified polygonal epithelium, and the first sign of the differentiation of the surrounding mesoderm which marks the position of the future external os (Bulmer, 1955) indicates that this epithelium lines the entire 'vaginal' portion of the canal, the uterine segment retaining its original columnar epithelium.

16½ *week foetus*

The crown–rump length of this specimen is unfortunately not known, but it shows a slightly later stage of development than the 112 mm. foetus. There is now a marked dorso-ventral elongation of the pars phallica and a relative and absolute shortening of the pars pelvina compared with the 94 mm. foetus. Though this change of shape has slightly modified the relative positions of the folds in the sinus wall their pattern remains essentially the same (Text-fig. 3).



Text-fig. 3. 16½ week foetus. Median sagittal section and the corresponding transverse sections through the sinus and the lower end of the utero-vaginal canal (×13½). The arrow at *s* indicates the level of the cranial tips of the sinus upgrowth, on either side of the lower end of the utero-vaginal canal. Other lettering and shading as for Text-fig. 1.

The sinus upgrowth extends cranially for a distance of about 1·6 mm.—approximately half the extent of the future vagina as indicated by the site of the external os. In most of its extent the upgrowth forms a solid transversely elongated epithelial plate, and cranially it extends for a short distance as bilateral wings on either side of the lower end of the utero-vaginal canal. Its epithelium is again differentiated into basal and superficial zones, and the thickness of section (12 μ) seems to accentuate the distinction between Müllerian and sinus epithelia (Pl. 2, fig. 11). The 'vaginal' portion of the utero-vaginal canal is lined by a four- or five-layered stratified Müllerian epithelium, and the superficial cells are now becoming more flattened.

At its caudal end the sinus upgrowth joins the enlargement of the sinus which lies cranial and dorsal to the urethro-vaginal folds, unsplit by any mesodermal septum, and this lower end of the upgrowth now shows three swellings of its epithelial plate, one centrally and one on either side. In these swellings, and in the dorsal

enlargement of the sinus, the epithelium is rather different from that in the cranial part of the sinus upgrowth. The internal cells are larger and the nuclei smaller, so that the distinction between basal and superficial zones is much more apparent (Pl. 2, fig. 12). In addition, particularly in the swellings of the sinus upgrowth, the cytoplasm of the internal cells is eosinophilic, and the cell walls deeply stained. The remainder of the pars pelvina, below and ventral to the urethro-vaginal folds, is still lined by an undifferentiated type of epithelium, consisting of four or five layers of small, darkly staining cells.

140 *mm. foetus*

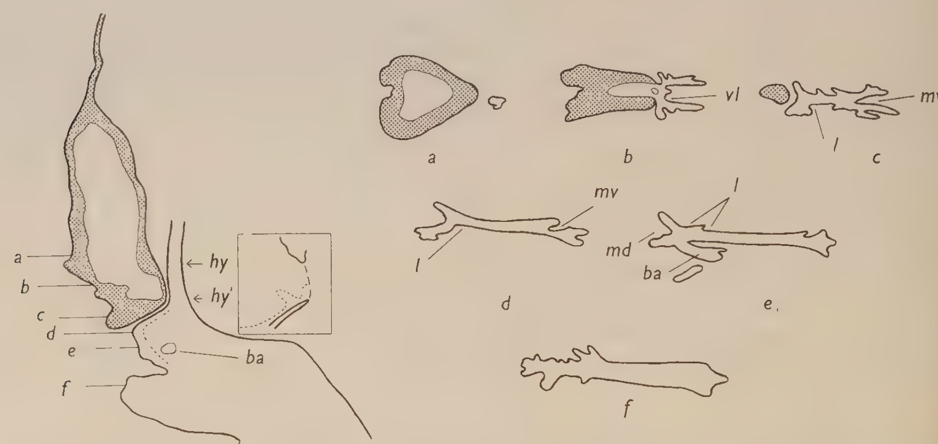
In this specimen many further developments are apparent. The sinus upgrowth extends to the lower end of the cervical canal as a solid, transversely elongated epithelial plate in which the basal and internal zones of cells can be distinguished. Here it meets a stratified squamous Müllerian epithelium (Pl. 2, fig. 13), which consists of very much smaller cells and lines the lower portion of the cervical canal immediately above the sinus upgrowth, intervening between this and the columnar epithelium of the rest of the uterus above. The vaginal fornices have not yet appeared.

As the sinus upgrowth is followed caudally, it develops, about the middle of its cranio-caudal extent, enlargements similar to those seen at the lower end of the upgrowth in the $16\frac{1}{2}$ week foetus—a large swelling centrally, and smaller swellings at each lateral extremity. Traced further caudally these swellings become larger, until eventually they merge together to form a single mass occupying about the lower one-third of the vagina, rather heart-shaped in cross-section and with a large central lumen (Text-fig. 4). In the swellings the vaginal epithelium consists of a narrow basal zone of darkly staining cells, with relatively large nuclei, and a mass of large internal pale-staining cells with very small nuclei, eosinophilic cytoplasm and very deeply stained cell walls (Pl. 3, fig. 14). In the canalized portion of the vagina the epithelium is very similar, with a thick zone of internal cells of which the superficial layers are flattened. The lumen is filled by a mass of desquamated material, and the PAS technique demonstrates that all but the basal cells of the epithelial lining are loaded with glycogen.

The lower end of the vagina communicates with the sinus by paired hymeneal orifices (Text-fig. 4*b* and Pl. 3, fig. 15), separated from each other by a small median mesodermal septum, and the great enlargement of the lower end of the vagina has resulted in the extension of its area of contact with the sinus, particularly on the lateral and caudal aspects of the orifices (Text-fig. 4*c*). In this way the hymen is formed, consisting of a plate of dense connective tissue, lined above by vaginal epithelium and below by the undifferentiated type of sinus epithelium which still occupies the upper part of the sinus (Pl. 3, fig. 17).

Associated with the increasing area of contact between the vagina and the sinus which gives rise to the hymen, the configuration of the sinus itself shows a considerable change from the stage represented by the $16\frac{1}{2}$ week foetus. The pars pelvina, as judged by the site of entry of the ducts of Bartholin's glands (Text-fig. 4), is now very short, and the original system of longitudinal folds, though it can still be followed, has become modified by the change in shape of this part of the sinus.

A striking feature, which seems to be associated with the invagination of the upper part of the dorsal wall of the sinus by the lower end of the vagina, is the accentuation of the upper end of the lateral fold, now lying parallel with the posterior portion of the hymen and separated from it by an upward extension of the dorso-lateral sinus bay—the bay which, at a lower level (Text-fig. 4), receives the ducts of Bartholin's glands. The dorso-lateral bay extends cranially and ventrally on the lower aspect of the hymen as far as the hymeneal orifices, where it terminates as the lateral fold becomes continuous with the hymeneal fold. In addition, it gives off a cranial diverticulum which ascends for a short distance on the lateral aspect of



Text-fig. 4. 140 mm. foetus. Median sagittal section through the vagina and the sinus, with the corresponding transverse sections ($\times 6\frac{2}{3}$). The stippling indicates the vaginal epithelium, and the vestibule is shown only in outline. Because of the dilatation of the upper end of the sinus it is not practicable to indicate the folds of its wall in the median sagittal section. However, the dorsal margin of the lower part of the lateral fold is shown by the dotted line, and the small inset figure shows the outline (dotted line) of the upper end of the dorso-lateral sinus bay and its cranial diverticulum in relation to the margins of the vaginal and sinus epithelia (continuous lines) and to the hymeneal orifices (interrupted line). *hy–hy'* indicates the level of the hymeneal orifices in relation to the median sagittal section. Other lettering as for Text-fig. 1. It will be seen that the change in the position of the lateral fold in this specimen, and to an even greater extent in the next (Text-fig. 5), differs from that described by Mijsberg (1924). The dorso-lateral bay is rotated relatively upwards and backwards with the downgrowth of the vagina into the sinus. According to Mijsberg, this bay is rotated upwards and forwards, an opinion for which the present material offers no evidence.

the lower end of the vagina (Pl. 3, figs. 15, 16), and presumably represents the cranial extremity of the dorso-lateral bay of the $16\frac{1}{2}$ week stage, maintaining its position despite the relative downgrowth of the lower end of the vagina.

The hymeneal folds, bounding the outer margins of the paired hymeneal orifices, represent the dorsal margins of the urethro-vaginal folds of the earlier stages. In the same way they form the boundary between the differentiated and undifferentiated types of sinus epithelium, and it is by the enlargement of the area of differentiated epithelium—now the lower end of the vagina—and its downgrowth relative to the urogenital sinus that the hymen is formed. The mesodermal septum between the two hymeneal orifices seems to differ in origin from the remainder of

the hymen, and probably results from the further development of a septum such as occurs in the 112 mm. foetus, splitting the root of the sinus upgrowth. By the enlargement and downgrowth of the lower end of the vagina the septum is projected forwards to divide the space between the dorsal margins of the urethrovaginal folds—in other words, to split the hymeneal orifice. The mesodermal septum of the 140 mm. stage is therefore lined on both its ventral and dorsal aspects by vaginal epithelium.

180 *mm. foetus*

The enlargement and canalisation of the vagina now extends throughout its entire length, and the fornices are well established. An important change is the disappearance of the zone of stratified squamous Müllerian epithelium from the lower portion of the cervical canal, and the sinus epithelium of the vagina meets the columnar epithelium of the uterus just inside the external os. Here there is a very clear line of distinction between the two epithelia (Pl. 3, fig. 18). Both give a strongly positive PAS reaction, granular in the vaginal epithelium and diffuse in the columnar cells of the cervix, but in the latter this reaction is not affected by previous salivary digestion.

The vagina is lined by a very thick stratified squamous epithelium (Pl. 3, fig. 19), in which it is possible to distinguish four cellular zones. The basal zone is formed by three or four layers of small cubical cells, with relatively large nuclei and basophil cytoplasm. The next zone consists of seven or eight layers of much larger cells, with relatively much smaller nuclei and pale, acidophil cytoplasm. The deeper layers of this zone consist of polygonal cells, with deeply stained cell walls, but the three or four superficial layers are formed by very flattened cells, with much thicker cell walls. The third zone consists of about ten layers of large, clear-staining polygonal cells, less flattened and with thinner cell walls than the most superficial cells of the second zone. The nuclei are very small, and are absent from many of the cells. The innermost zone is formed by four or five layers of cells, similar to those of the third zone but more flattened. Many of the cells are without nuclei, and the nuclei which are present are very small and pyknotic.

Glycogen is extremely abundant in the vaginal epithelium in all but the basal zone of cells, as shown by staining with Best's ammoniacal carmine and by the PAS technique. It is generally located as large granules, sometimes almost filling the cytoplasm of the cell, and is usually, but not always, restricted to the part of the cell on the proximal side of the nucleus. In addition, the cell walls of the three superficial zones show a positive PAS reaction after previous salivary digestion, similar to that demonstrated by Wislocki, Fawcett & Dempsey (1951) in the vaginal epithelium of the adult.

A further development from the 140 mm. stage is the lining of the under surface of the hymen and the adjacent portion of the vestibule by an epithelium similar to the vaginal epithelium, though rather thinner. Associated with the further relative downgrowth of the lower end of the vagina, and the resulting more horizontal position of the hymen (Text-fig. 5), the pars pelvina has almost disappeared. In fact, the shape of the sinus is so distorted from its original form that the distinction between pars pelvina and pars phallica now has little meaning.

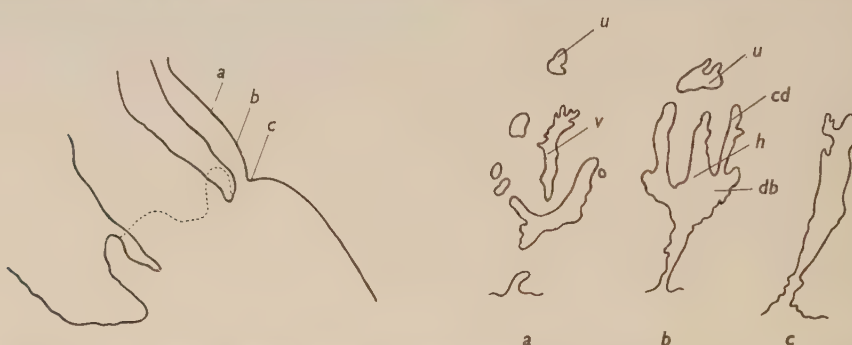
By this stage the sinus extends further dorsally in relation to the under surface of the hymen, and the dorso-lateral sinus bays are very prominent, forming a dilatation of the vestibule immediately beneath the hymen (Text-figs. 5*a*, *b*). They extend further ventrally than in the 140 mm. foetus, reaching forwards around the sides of the anterior portion of the hymen. As before, they send short diverticula cranially, on either side of the lower end of the vagina. The hymenal orifice is unsplit.

200 *mm. foetus*

This specimen shows little further change, but the sagittal plane of section gives a very clear picture of the penetration of the lower end of the cervical canal by the vaginal epithelium, to a distance of about 3 mm. above the external os.

375 *mm. foetus*

The vaginal epithelium is now considerably thinner, but the lumen is filled with cell debris, suggesting that the decrease in thickness may be largely due to desquamation of the superficial cell layers. Only three cellular zones can be distinguished (Pl. 3, fig. 20). In the basal zone there are usually three layers of small cubical cells, with large nuclei and basophil cytoplasm. The intermediate zone is



Text-fig. 5. 180 mm. foetus. Median sagittal section through the lower end of the vagina and the vestibule, with the corresponding coronal sections (× 4). The dotted line in the sagittal section shows the relative extent of the dorso-lateral bays. The coronal sections are shown with their cranial ends towards the top. *u*, urethra; *v*, vagina; *h*, hymeneal orifice; *db*, dorso-lateral sinus bay; *cd*, cranial diverticulum of dorso-lateral sinus bay.

formed by four or five layers of much larger polygonal cells, with relatively smaller nuclei and deeply staining cell walls. Superficially there are two or three layers of large flattened cells with small pyknotic nuclei. The superficial and intermediate zones are not clearly distinct from each other, and both are rich in glycogen granules. It is possible that these two zones represent the second zone of the epithelium of the 180 mm. foetus, the third and fourth zones having been desquamated. Again the cell walls give a positive PAS reaction after salivary digestion.

The sinus shows the further progress of the developmental changes noticed at the 180 mm. stage. The dorso-lateral bays extend still further forwards, now reaching around the urethral orifice, and the dorsal extension of the sinus in relation to the under surface of the hymen is still more marked. The whole of the upper part of the

sinus is lined by an epithelium similar to that of the vagina, and now of about the same thickness.

1 *month infant*

Little change is noticed in this specimen, except in the character of the vaginal epithelium (Pl. 3, fig. 21). This is considerably thinner than in the later foetal stages and consists mainly of polygonal cells with relatively large nuclei, deeply staining cytoplasm and indistinct cell walls. Glycogen is no longer present, and the cell walls no longer give a positive PAS reaction.

DISCUSSION

There can be little doubt that the epithelium of the human vagina is entirely derived from an upgrowth of sinus cells, as Vilas (1932), Kempermann (1935) and Meyer (1934–38) claimed, but the manner of formation of this upgrowth does not seem to be completely in accord with their findings. Vilas believed that the epithelium in the dorsal wall of the sinus proliferated in two different ways. An inner proliferation of pale-staining cells grew ventrally and medially in the internal layers of the dorsal sinus wall, meeting its fellow to form a crest which projected ventrally into the sinus lumen. An outer proliferation of darkly staining cells grew dorsally, displaced the Müllerian cells from the Müllerian tubercle and extended cranially as an epithelial plate, subsequently canalized to form the vagina. The views of Meyer and Kempermann were essentially similar.

It is interesting to compare this with Koff's account (1933) of the origin of the 'sino-vaginal bulbs', evaginations of the dorso-lateral aspect of the sinus on each side. Koff found that their epithelium was different from that of the rest of the sinus, consisting of well-marked basal and superficial zones, the basal cells darkly staining and the superficial cells pale-staining. The bulbs became larger and eventually fused with each other to form a solid cellular mass, which displaced the Müllerian tissue dorsally and cranially and eventually became canalized as the lower one-fifth of the vagina. The sino-vaginal bulbs seem to be the structures which have here been termed the dorso-lateral projections. In the same way they were joined by the lower ends of the Wolffian ducts, when these persisted, and carried the attachments of the ducts with them as they extended cranially. Koff did not mention any proliferation of darkly staining cells from the intermediate portion of the dorsal wall of the sinus, such as occurs in the 65 mm., 68 mm. and 14-week foetuses of this present collection, and would presumably regard this proliferation as an early stage in the fusion of the sino-vaginal bulbs.

Up to a point, the findings of this present investigation combine both these views on the initial origin of the sinus contribution to the vagina, the sinus upgrowth taking origin from all three of the elements associated with the activity of the dorsal wall of the sinus, first noticed in the 65 mm. foetus. The junction of the persistent lower end of the left Wolffian duct with the sinus upgrowth in the 94 mm. specimen indicates that, at any rate in this foetus, the dorso-lateral projection must have participated in the formation of the upgrowth. On the other hand, in the 65 mm. foetus there seems no doubt as to the entity of the intermediate proliferation of darkly staining cells, and their contribution to the sinus

upgrowth cannot be ignored. Only by the fusion of the three initial elements, by the 94 mm. stage, is the single sinus upgrowth produced. It is interesting that the formation of the sinus upgrowth in the human foetus bears such a close similarity to the initial origin of the lower vaginal segment in the sheep (Bulmer, 1956). There may be no great significance in the distinction between the dorso-lateral projections and the intermediate proliferation of darkly staining cells, all three representing a continuous cellular proliferation from this area of the sinus wall. There seems little doubt, however, of the identification of the dorso-lateral projections with Koff's sino-vaginal bulbs, and of the intermediate proliferation with the outer sinus proliferation of Vilas.

In many instances, it appears, the root of the sinus upgrowth is split at its origin from the dorsal wall of the sinus by the inclusion of a small mesodermal septum. Vilas pointed out that in his early foetuses, from the 38 mm. stage onwards, a small mass of mesoderm interrupted the contact between the Müllerian epithelium and the dorsal wall of the sinus in the midline, and this has been noticed here in the 28, 42 and 50 mm. foetuses. Vilas described the initial bilateral origin of the darkly staining sinus proliferation, the two origins then fusing to form a single sinus upgrowth. In the 65 mm. foetus, described here, there is no sign of such a bilateral origin, but in the 68 mm. foetus the proliferation is split by a small mesodermal septum. The inclusion of a mesodermal septum in the root of the sinus upgrowth is most probably a result of the persistence of such a septum at the Müllerian tubercle, and this would suggest that the darkly staining proliferation only arises where the sinus is actually in contact with Müllerian cells. The persistence of such a septum, as in the 112 mm. foetus, and its development into a hymeneal septum, as in the 140 mm. foetus, must be fairly common, and occurred in many of the specimens examined by Meyer (1934–38). The persistence of a more extensive septum in the utero-vaginal canal would account, in a similar manner, for a congenital duplication of the vagina.

The further development of the sinus upgrowth, after the 94 mm. stage, is of some interest. Vilas found that as the Müllerian epithelium receded before the advancing plate of sinus epithelium the lower end of the utero-vaginal canal, including its whole 'vaginal' portion, became completely occluded by the proliferation of its lining cells. A solid vaginal plate was thereby formed, composed of Müllerian tissue above and sinus tissue below, but the sinus epithelium gradually extended further and further cranially until eventually the Müllerian epithelium was completely displaced from the vaginal plate. In the series of foetuses examined here the occlusion of the lower end of the utero-vaginal canal is not a prominent feature. It is first seen at the 65 mm. stage and persists until the Müllerian epithelium is completely excluded from the vagina, but it never involves more than a short segment. It is to be noted, however, that the stratification of the epithelium of the utero-vaginal canal does extend, apparently by the 94 mm. stage, throughout the entire 'vaginal' portion of the canal.

Koff also described a 'primitive vaginal plate', formed in its lower portion by the solid epithelial mass of the sino-vaginal bulbs, and in its upper portion from the occlusion of the 'vaginal' portion of the utero-vaginal canal by the proliferation of its lining cells. In this solid vaginal plate Koff claimed that he was able to distinguish histologically between the sinus and Müllerian components, and only after

the 142 mm. stage was this distinction no longer apparent. He assumed that the relative proportions of the two epithelia remained unchanged after this stage, the sinus epithelium being restricted to the lower one-fifth of the vagina.

Unfortunately, Koff showed no microphotograph of the epithelial distinction between the components of his primitive vaginal plate, but the criterion which he used for the identification of the sinus epithelium was its differentiation into external darkly staining and internal pale-staining zones. It has been noticed in the series of foetuses described here that after the 94 mm. stage the internal cells in the cranial part of the sinus upgrowth are much less pale-staining than they are further caudally. For instance, in the 16½ week foetus the large, markedly pale-staining cells are restricted to the enlarged portion of the dorsal wall of the sinus and to the swellings at the caudal end of the sinus upgrowth. Indeed, Koff seems to have identified the two laterally placed swellings with the sino-vaginal bulbs themselves—a conclusion which is difficult to follow and for which there is no evidence in the material described here. The epithelium at the cranial end of the sinus upgrowth is of the same essential character as that further caudally. There is no evidence of any sharp histological distinction in the vaginal mass except the one which has been identified as the junction of the cranial end of the sinus upgrowth with the caudal end of the Müllerian tissue. Koff's description offers no explanation for the very marked distinction between the two, occurring about half-way up the 'vagina' in the 16½ week foetus.

The most likely explanation for the histological differences between the cranial and caudal ends of the sinus upgrowth is that the differentiation of its cells progresses from below upwards. Thus, the cells of the caudal end of the upgrowth in the 112 mm. and 16½ week foetuses, when the internal cells are larger, their nuclei smaller and the cell walls deeply stained with eosin, are similar to those occupying the whole of the lower half of the vagina in the 140 mm. foetus. The transversely elongated plate of the vagina appears to differentiate, at each particular stage, by first enlarging to form swellings. The epithelium correspondingly proliferates and the internal cells become large, with small nuclei and deeply stained cell walls. The swellings become still larger, and confluent with each other, the central cells then desquamating to form the vaginal lumen. The 140 mm. foetus shows later and later stages of this process as the sinus upgrowth is followed further and further caudally. The vaginal epithelium continues to proliferate, presumably under the influence of hormonal stimulation, and it is interesting to compare its structure in the two older foetuses with the adult vaginal epithelium as described by Papanicolou, Traut & Marchetti (1948), and more particularly, in view of the probable hormonal influences at work, with the vaginal epithelium of the pregnant woman, described by Smith & Brunner (1934).

As indicated above, many earlier workers have denied the participation of sinus epithelium in the formation of the vagina. Berry Hart (1896, 1901 and 1911) and Mijsberg (1924) described a contribution to the vagina from the 'Wolffian bulbs', proliferations of the epithelium of the lower ends of the Wolffian ducts. There seems no doubt that the Wolffian bulbs were the dorso-lateral projections, and there is no evidence that these latter arise from Wolffian epithelium. Their cells are unlike the cubical Wolffian cells which lie dorsal to them, and much more closely

resemble those of the dorsal sinus wall. Berry Hart believed that the epithelium of the Wolffian bulbs extended throughout the entire vagina, while Mijsberg, in a sense less correctly, restricted the Wolffian contribution to a lower vaginal segment. His reasons for this were similar to those which influenced Koff to believe that the sino-vaginal bulbs formed only the lower end of the vagina.

Other authors have held to the view that the human vagina is entirely derived from the Müllerian utero-vaginal canal. Bloomfield & Frazer (1927) illustrated a section rather similar to the ones shown in Pl. 1, figs. 4, 5 and 6, for the 65 mm. foetus, but their interpretation was very different, as they believed that the pale-staining cells in the dorsal sinus wall were the Müllerian cells breaking through into the sinus at the Müllerian tubercle. It is clear that the findings in the 65 mm. foetus make such a view untenable, and this misinterpretation presumably led Bloomfield & Frazer to think that the whole extent of the differentiated type of sinus epithelium was of Müllerian origin.

While the sinus origin of the vaginal epithelium appears to be established, one or two points of detail merit further consideration. From the earliest stage examined, represented by the 28 mm. foetus, the epithelium which lines the part of the sinus from which the sinus upgrowth later arises differs from that of the rest of the pars pelvina in consisting of two cellular zones—darkly staining cells deeply and pale-staining cells superficially. So far, it has been tacitly assumed that both these cell types are of sinus origin, but other workers have placed different interpretations upon them. Vilas (1932) believed that the darkly staining cells were of sinus origin, but was prepared to accept that the pale-staining cells were Wolffian, growing ventrally and medially in the dorsal wall of the sinus to form the ventrally projecting *Wolffsche Kamm*, identified by Kempermann (1931) and evident in the 65 mm. foetus of this present investigation. Kempermann (1935) also believed that the pale-staining cells were Wolffian in origin, but that they later completely disappeared. The pale-staining cells in the internal layers of the sinus upgrowth at a stage such as that represented by the 94 mm. foetus of this collection were not the Wolffian cells, but a new generation of cells derived from the sinus. Meyer (1934–38), however, believed that the pale-staining Wolffian cells did persist into later foetal life, identifying them with the large pale-staining cells such as occur at the root of the sinus upgrowth in the 112 mm. and $16\frac{1}{2}$ week foetuses.

This question does not seem possible of solution by ordinary histological methods. Nevertheless, the pale-staining cells in the dorsal wall of the sinus from the 28 mm. stage onwards bear no obvious similarity to the cubical cells which line the Wolffian ducts, and are distinct from the Wolffian cells which lie dorsal to them. It appears just as reasonable to suppose that the pale-staining cells are derivatives of the darkly staining basal cells, and that there is no incorporation of Wolffian cells in the dorsal wall of the sinus at this stage. No such ingrowth occurs in other mammals, and it seems unnecessary to postulate it in the human foetus. Nevertheless, it must be admitted that the differentiation of the sinus epithelium from which the sinus upgrowth arises is a remarkable feature, and it too has not been described in other mammals.

Also of some significance is the problem of the behaviour of the lower ends of the Wolffian ducts during the development of the vagina. Descriptions of the lower ends

of the Wolffian ducts in female foetuses fall generally into two main categories. In the first are those which maintain that the Wolffian ducts continue to open into the sinus at their original site, immediately lateral to the position of the Müllerian tubercle. Thus, Meyer (1909) described the persistence of the lower ends of the Wolffian ducts in a large number of older foetuses and new-born children, running into the substance of the hymen and opening into the vestibule close beside the hymeneal orifice. On the other hand, many other workers (Tourneux & Legay, 1884; van Ackeren, 1889; Mijsberg, 1924; Koff, 1933) have described the cranial migration of the lower ends of the Wolffian ducts to join the lower end of the vagina itself, though the ducts degenerated very soon after and did not persist into late foetal life.

At first sight, the present investigation supports this latter view. In the 94 mm. foetus the lower end of the left Wolffian duct joins the sinus upgrowth, indicating that the dorso-lateral projection has carried the Wolffian remnant with it as it grew cranially, while in none of the older foetuses was there any trace of the lower ends of the Wolffian ducts. Nevertheless, this evidence is insufficient to suggest that the lower ends of the Wolffian ducts never maintain their openings into the sinus beside the original site of the Müllerian tubercle, particularly in view of the large number of such cases described by Meyer (1909). At that time Meyer regarded these Wolffian remnants as indicative of the entirely Müllerian origin of the vagina, but in his later publications (1934–38) pointed out that they were equally in accord with the account of vaginal development given by Vilas. The Wolffian ducts were not involved in the proliferation which Vilas believed to form the epithelial plate of the vagina, and were therefore left behind near the hymen. Nevertheless, the structures which Meyer now identified as persistent Wolffian remnants do not appear to occupy the same site as those which he had described earlier, but join the lower end of the vagina on the upper aspect of the hymen. In other words, they have been carried cranially for a short distance during the formation of the sinus upgrowth.

It may be that in some instances the lower ends of the Wolffian ducts are not carried cranially with the sinus upgrowth to such a great extent as they have been in the 94 mm. foetus, or indeed that they are not carried cranially at all. This may be because the dorso-lateral projections do not play such a large part in the formation of the sinus upgrowth in these cases, or because the manner of their growth is such as not to involve the lower ends of the Wolffian ducts. In other words, it is possible, as von Lippmann suggested (1939), though with rather a different purpose, that there is some variability in the behaviour of the lower ends of the Wolffian ducts during the early stages of vaginal development. This is supported by the fact that an ectopic ureter, presumably opening by a persistent Wolffian remnant, may join either the vagina or the vestibule (Kermauner, 1909). It is also possible that many of the structures which have been identified as persistent Wolffian remnants were, in fact, not so. In the 140 mm. foetus the dorso-lateral sinus bays end above by sending short diverticula upwards on either side of the lower end of the vagina, lined by the undifferentiated type of sinus epithelium. In the 180 mm. foetus these diverticula are still lined by the undifferentiated epithelium, though the dorso-lateral bays themselves are occupied by an epithelium very similar to that of the vagina. The diverticula are presumably associated with the relative downgrowth of the lower end of the vagina, as has already been pointed out. They are, however,

very similar to the 'Wolffian remnant' described by Bloomfield & Frazer (1927), in a 170 mm. foetus of their collection, but their lining epithelium leaves no doubt of their origin (Pl. 3, fig. 16).

An interesting viewpoint on the development of the vagina was suggested by Zuckerman (1940), who believed that any epithelium of the adult genital tract which responded to oestrogenic stimulation by a stratified squamous proliferation—a 'squamous response'—was a derivative of the sinus epithelium of the foetus. The conclusion of this present investigation, that the entire vaginal epithelium is derived from the sinus upgrowth, might appear to support Zuckerman's hypothesis. There is, however, a considerable body of embryological evidence which indicates that in many other mammalian forms the upper vaginal segment, though it is lined by a stratified squamous epithelium and gives a squamous response to oestrogenic stimulation, is a Müllerian derivative. Thus, it seems likely that when the Müllerian epithelium does persist in the vagina it tends to be of stratified squamous form and its response to oestrogenic stimulation squamous in type. Of some interest in this respect is the lining of the 'vaginal' portion of the utero-vaginal canal in the human foetus by a stratified squamous epithelium of Müllerian origin, and the disappearance of this epithelium after the 140 mm. stage, when it is displaced into the cervical canal. In the absence of any firm knowledge of the hormonal conditions in female human foetuses, we do not know how this stratified Müllerian epithelium responds to oestrogenic stimulation. Probably, however, there is some controlling factor, producing its effect in foetal life in the human subject and throughout life in many other mammals, which stimulates Müllerian cells in the vagina, but not in the uterus, to form a stratified squamous epithelium.

One of the most interesting problems is that of the extent to which the development of the human vagina is influenced by hormonal factors. It is a reasonable supposition that the enormous activity of the vaginal epithelium in the older foetuses is a result of stimulation by maternal oestrogens, as Fraenkel & Papanicolou (1938) suggested. The gross differences between the vaginal epithelium in these foetuses and that of the 1-month old infant imply that this oestrogenic stimulation must extend over a considerable period of foetal life—at any rate from the 112 mm. stage onwards. The question of the hormonal control of genital development has introduced a new field of experimental embryology, in which a very large amount of work has already been carried out (for bibliography see Jost, 1948), and it raises many interesting problems concerning the development of the human vagina. It may be that the enormous enlargement of the lower end of the vaginal mass at the 140 mm. stage, associated with the formation of the hymen, is a result of hormonal stimulation to which only the differentiated type of sinus epithelium is sensitive. At an earlier stage, the extension of the sinus upgrowth itself may be due to a similar selective response of the differentiated epithelium. Some of the marked differences between vaginal development in man and in other mammals may result from differences in the degree of hormonal stimulation at various critical periods of foetal life, rather than from inherent differences in the structures which go to form the vagina. On the other hand, the histological differentiation of the epithelium of the sinus upgrowth in the human foetus may be associated with an increased sensitivity to hormonal stimulation, compared with other mammalian forms. While

such suggestions can be only tentative, it is, nevertheless, very likely that the endocrinology of vaginal development holds the key to many of these outstanding problems.

SUMMARY

1. The development of the human vagina has been studied from a series of female foetuses ranging from 28 to 375 mm. crown–rump length, and from a 1-month old infant.

2. An upgrowth arises from an area of 'differentiated' epithelium in the dorsal wall of the sinus, in a manner which might be said to combine the descriptions of Vilas (1932) and Koff (1933).

3. The sinus upgrowth extends throughout the entire region of the vagina by the 140 mm. stage, and forms the whole of its epithelial lining. The changes in the vaginal epithelium of older foetuses are also described.

4. The findings are discussed in relation to the question of an early ingrowth of Wolffian epithelium into the dorsal wall of the sinus, the problem of the behaviour of the lower ends of the Wolffian ducts in female foetuses, and recent opinions on the significance of the sex hormones in genital development.

My thanks are due to Prof. J. D. Boyd, for the loan of most of the material which I was able to study. I am also indebted to Messrs J. F. Crane and A. Cain for the microphotographs, and to Mr W. Cruickshank for his assistance with the diagrams.

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EXPLANATION OF PLATES

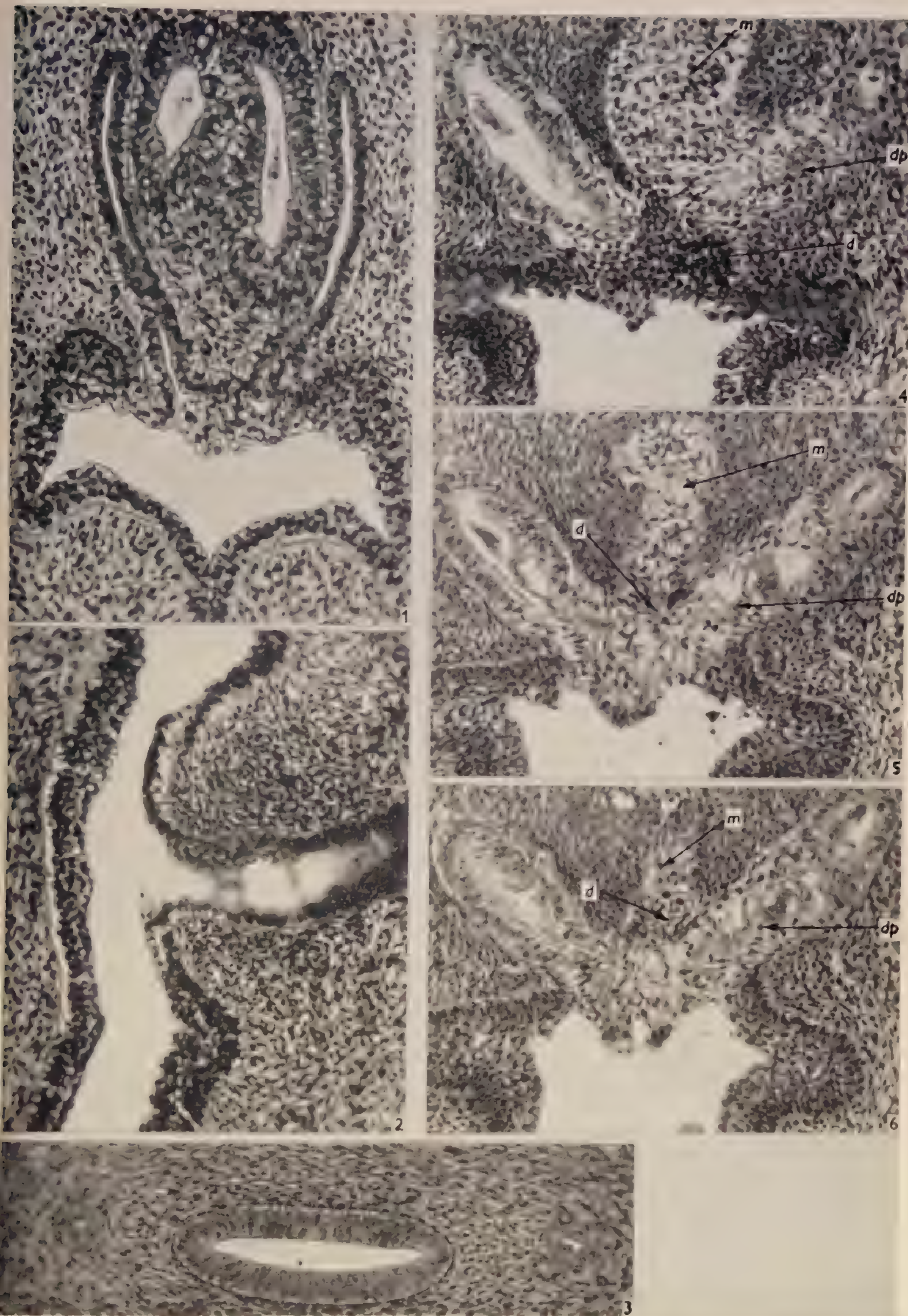
PLATE 1

Fig. 1. 28 mm. embryo. Transverse section through the openings of the Wolffian ducts into the urogenital sinus. The sinus epithelium consists of basal deeply staining and superficial pale-staining cells, and the pale-staining cells extend for a short distance into the lower ends of the Wolffian ducts. The Müllerian ducts are in close relation with the Wolffian ducts, but a small mass of mesoderm separates them from the sinus. (×215.)

Fig. 2. 48 mm. foetus. Sagittal section through the opening of the right Wolffian duct. The differentiation of the sinus epithelium in this region can be seen, and the extension of the pale-staining cells into the lower end of the Wolffian duct. (×215.)

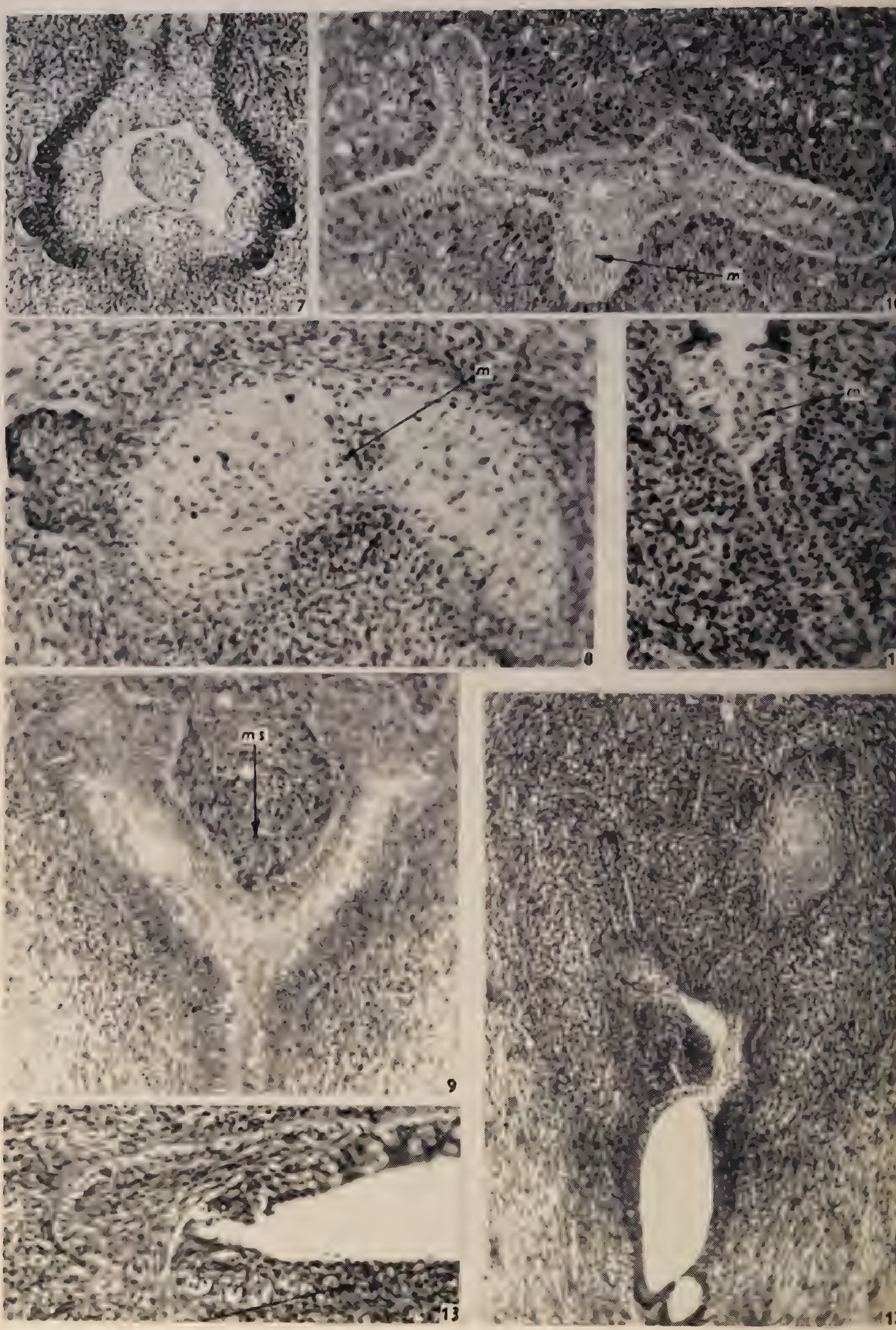
Fig. 3. 65 mm. foetus. Transverse section through the genital cord. The Wolffian ducts are very small structures, on either side of the utero-vaginal canal. (×215.)

Fig. 4. 65 mm. foetus. Transverse section through the junction of Müllerian and sinus epithelia. A mass of darkly staining cells (*d*), arising from the dorsal wall of the sinus, separates the caudal end of the Müllerian epithelium (*m*) from the sinus lumen. On the right side the section passes below the bulk of the dorso-lateral projection, and the Wolffian duct is large. On the left the Wolffian duct is a small structure, applied to the dorsal aspect of the dorso-lateral projection (*dp*). (×215.)

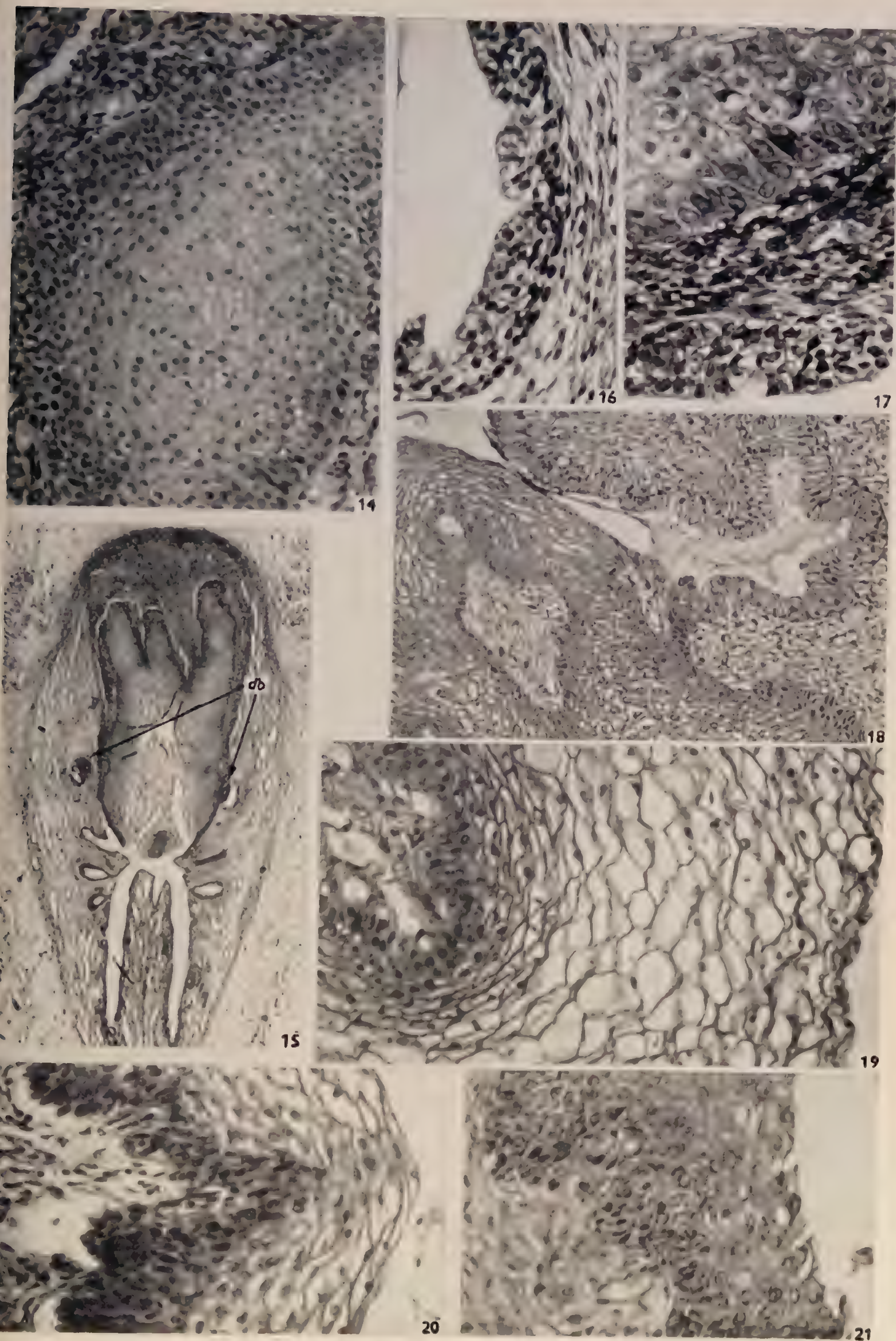


BULMER—THE DEVELOPMENT OF THE HUMAN VAGINA

(*Facing p.* 508)



BULMER—THE DEVELOPMENT OF THE HUMAN VAGINA



BULMER—The development of the human vagina

Fig. 5. 65 mm. foetus. Transverse section 16 μ caudal to Fig. 4. The Müllerian epithelium now forms a smaller mass dorsally, and a few of the darkly staining sinus cells separate it from the pale-staining sinus epithelium ventrally. ($\times$215.)

Fig. 6. 65 mm. foetus. Transverse section 8 μ caudal to Fig. 5. ($\times$215.)

PLATE 2

Fig. 7. 94 mm. foetus. Coronal section through the cranial and dorsal enlargement of the sinus which forms the root of the sinus upgrowth. The epithelial differentiation is very well marked. ($\times$84.)

Fig. 8. 94 mm. foetus. Coronal section through the sinus upgrowth, 90 μ dorsal to Fig. 7. The small darkly staining cells in the centre of the section (*m*) represent the caudal tip of the Müllerian epithelium. A thin layer of darkly staining cells forms the outer layer of the sinus upgrowth, and there is a small mass of these cells on the right (to the left of the photograph). 90 μ further dorsally, a similar small mass, on the other side, is joined by the persistent lower end of the left Wolffian duct. ($\times$215.)

Fig. 9. 112 mm. foetus. Transverse section through the dorsal part of the sinus, showing the two roots of the sinus upgrowth separated from each other by the mesodermal septum (*ms*). The pale-staining internal cells of the sinus are very prominent. ($\times$215.)

Fig. 10. 112 mm. foetus. Transverse section through the vaginal mass, 288 μ cranial to Fig. 9. The sinus upgrowth forms two lateral wings, while the central portion (*m*) is formed by the paler-staining polygonal cells of the caudal end of the Müllerian tissue. ($\times$215.)

Fig. 11. 16½ week foetus. Transverse section through the junction of the Müllerian epithelium of the utero-vaginal canal (*m*) with the cranial wing of the sinus upgrowth on the left side. ($\times$215.)

Fig. 12. 16½ week foetus. Transverse section through the sinus showing the junction of its dorsal wall with the caudal end of the sinus upgrowth. The central enlargement of the upgrowth is immediately dorsal to the sinus lumen, and a further enlargement projects dorsally from it. The large clear-staining internal cells are prominent in the central enlargement. In the dorsal enlargement the cytoplasm and cell boundaries of the internal cells are markedly eosinophilic. ($\times$84.)

Fig. 13. 140 mm. foetus. Transverse section through the left side of the cervical canal, showing the junction between the stratified Müllerian epithelium (*m*) and the cranial tip of the sinus upgrowth. ($\times$160.)

PLATE 3

Fig. 14. 140 mm. foetus. Transverse section through the left vaginal enlargement, just below the middle of the cranio-caudal extent of the vagina. The internal cells are large, their nuclei relatively small and the cell-boundaries markedly eosinophilic. ($\times$215.)

Fig. 15. 140 mm. foetus. Transverse section through the urogenital sinus and the lower end of the vagina, at the level of the paired hymeneal orifices. On each side of the ventro-lateral aspect of the vagina is the small cranial extremity of the dorso-lateral sinus bay (*db*). ($\times$30.)

Fig. 16. 140 mm. foetus. Transverse section through the wall of the dorso-lateral sinus bay, showing its lining of undifferentiated sinus epithelium. ($\times$450.)

Fig. 17. 140 mm. foetus. Transverse section through the lower portion of the hymen, showing the vaginal epithelium above, separated by the hymeneal connective tissue from the undifferentiated sinus epithelium below. ($\times$450.)

Fig. 18. 180 mm. foetus. Transverse section through the junction of the vaginal epithelium with the columnar epithelium of the cervix. ($\times$120.)

Fig. 19. 180 mm. foetus. Section through the vaginal epithelium. ($\times$280.)

Fig. 20. 375 mm. foetus. Section of the vaginal epithelium overlying a mesodermal papilla. ($\times$280.)

Fig. 21. Vaginal epithelium in the 1-month old infant. The epithelium is not of uniform thickness, and this photograph is taken from an area where it is rather thicker than usual, in order to give a better impression of its histological structure. ($\times$320.)

THE DEVELOPMENT OF CERTAIN HUMAN DURAL VENOUS SINUSES

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Although Streeter's (1915, 1918) accounts of the development of the human dural venous sinuses have become widely accepted, a critical analysis of these papers suggests that certain contained morphological interpretations are incorrect; in particular, those concerning the superior petrosal and petro-squamous sinuses and the post-glenoid vein.

Rathke (1838) believed that the brain was primarily drained by an external jugular vein which left the skull through a foramen between the temporo-mandibular joint and the tympanic ring, and that later a new vein, the internal jugular, grew cranialwards out of the external jugular and entered the skull by the jugular foramen. Only in man and apes did this new route receive all the intra-cranial venous outflow, lower mammals retaining the primary route to a variable degree. Luschka (1862) held similar views, and named the cranial exit of the external jugular vein the foramen jugulare spurium, and described (1867) its occasional occurrence in adult human temporal bones. Kölliker (1879) confirmed Luschka's interpretations.

Salzer (1895) described dural venous sinus development in man, rabbit, guinea-pig, pig and cat, finding in all a primary head vein consisting of vena capitis lateralis and vena capitis medialis. All species showed a similar mode of dural sinus development therefrom and two important observations were recorded:

(1) The vena capitis lateralis ran in the petrous temporal bone in company with the post-ganglionic portion of the facial nerve.

(2) The post-glenoid vein developed as a secondary connexion between the transverse sinus and the external jugular vein and co-existed with vena capitis lateralis. Grosser (1901) recorded the same mode of development of the head veins in bats.

Hochstetter (1896) found a typical primary head vein in embryonic Monotremes and here, also, the vena capitis lateralis ran in the facial nerve canal. In both adult *Tachyglossus* and *Ornithorhynchus* a petro-squamous sinus entered the anterior end of the facial nerve canal and emerged through the stylomastoid foramen and a persistent vena capitis lateralis joined the *internal* jugular vein. Neither genus manifests a post-glenoid vein. Later, however, he stated (1906) that the vena capitis lateralis left the skull between the nerves V and VII and his ill-worded statement has caused much confusion regarding the relation of the vena capitis lateralis ~~to the~~ post-glenoid vein.

Mall's (1905) account of the development of the human dural venous sinuses is difficult to follow, but he recognized a middle cerebral vein, joining the junction of

* Preliminary work carried out at St Bartholomew's Hospital Medical College, London.

venae capitis medialis and lateralis, which became the superior petrosal sinus: the petro-squamous sinus was ignored. Mall regarded the vena capitis lateralis as being clearly outside the skull, emerging therefrom between the nerves V and VII, along the root of the latter, in line with the foramen jugulare spurium, and communicating with the internal jugular vein.

Markowski (1911, 1922) showed that the human embryonic transverse sinus divided into the sigmoid sinus, joining the internal jugular vein, and the pro-otic vein, entering the junction of vena capitis medialis and vena capitis lateralis immediately caudal to the trigeminal ganglion. He described the pro-otic vein, following the regression of the vena capitis lateralis, as a continuation to the transverse sinus of the vena capitis medialis, and as having, in older embryos, a situation identical with that of the petro-squamous sinus of the adult: the middle meningeal veins were formed from tributaries of the pro-otic vein: the superior petrosal sinus was derived from a small cerebral vein. Streeter (1915, 1918) could not confirm Markowski's account of the superior petrosal sinus and was unconvinced of its identity with the pro-otic vein. Streeter himself regarded the 'foramen jugulare spurium' of the adult skull as the exit of a decadent vena capitis lateralis. The issue was further confused by van Gelderen (1925), who stated that the persistent middle cerebral vein of aplacental mammals was known as either the pro-otic vein or the anterior branch of the transverse sinus in the placental mammals, but in Primates as the superior petrosal sinus. According to this author, the vena capitis medialis, occupying the cavum epiptericum, is intracranial and the vena capitis lateralis, overhung by the otic capsule, is extracranial. But the cavum epiptericum is an extracranial space containing the ganglia of V and VII and traversed by nerve VI, the head vein and the stapedial artery (de Beer, 1937). Boyd (1930) questioned Streeter's interpretation of the post-glenoid vein as a persistent vena capitis lateralis but offered no alternative explanation.

In the rat the main intracranial venous outflow is by the post-glenoid vein, shown (Butler, 1953) not to be a persistent vena capitis lateralis; the pro-otic vein becomes the petro-squamous, not the superior petrosal sinus.

In view of my own findings concerning the relationship of the pro-otic vein to the vena capitis lateralis and vena capitis medialis in the rat it was decided to re-investigate the development of the human dural venous sinuses.

MATERIAL AND METHODS

Twelve human embryos ranging from 7·5 to 30·0 mm. crown-rump (C.R.) length were examined, and graphic reconstructions of the head veins were made of seven. Serial sections of eight foetal heads ranging from 42·0 to 175·0 mm. C.R. length were examined and lateral graphic reconstructions of the head veins made from two of them. The engorged veins in the head of a foetus of 85·0 mm. C.R. length were dissected. The pattern of endocranial vascular grooving was examined in thirty adults. The cavernous sinus was examined in one child of 8 years and in fifteen adults between 27 and 80 years old.

OBSERVATIONS

A. *The embryonic period*, 28–48 *days*

Stage 1. 7·5 *mm. crown-rump length* (Fig. 1)

Anterior cerebral, maxillary and pituitary veins unite, deep to ganglion V, forming the vena capitis medialis. The latter vein turns lateralwards around the caudal margin of ganglion V and, just deep to the ectoderm, turns caudalwards as the vena capitis lateralis which runs lateral to the otic vesicle and to ganglia VII, IX and X. Caudal to ganglion X it turns through 90 degrees to continue as the anterior cardinal

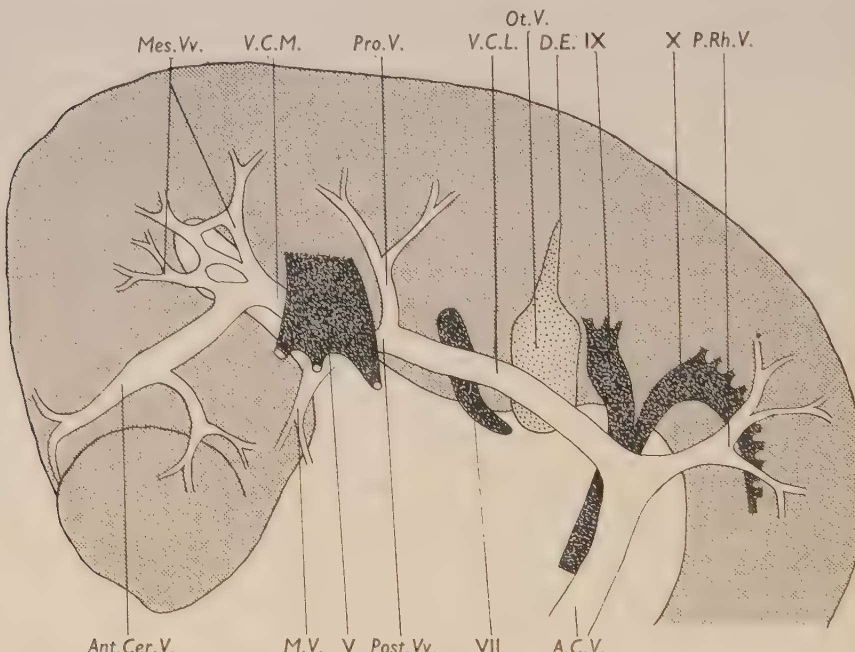

Fig. 1. Left lateral reconstruction of the head veins in a 7·5 mm. C.R. length embryo. The meaning of abbreviations for all figures are given on p. 526.

vein. The laterally directed part of the primary head vein running surfacewards between ganglia V and VII will be here called the post-trigeminal vein. It receives a large pro-otic vein into its dorsal surface. A large posterior rhombencephalic vein enters the junction of vena capitis lateralis and anterior cardinal vein caudal to ganglion X.

Stage 2. 14·0 *mm. crown-rump length* (Fig. 2)

The primary head vein is constituted as in the previous embryo but its tributaries are more complex. The commencement of the anterior cerebral veins, lying between the cerebral hemispheres, are coalescing to form a plexiform, midline channel, the superior sagittal sinus. Immediately anterior to the pineal evagination the two anterior cerebral veins diverge to run along the caudal margin of the cerebral hemisphere before crossing the lateral aspect of the midbrain to join the vena capitis

medialis. Each receives dorsally numerous mesencephalic veins and ventrally small cerebral veins and a lateral diencephalic vein.

Prior to passing medial to the anterior margin of ganglion V each anterior cerebral vein passes between nerves III and IV, just below their crossing point.

A small peri-trigeminal vein runs forwards across the lateral surface of ganglion V joining the termination of the anterior cerebral vein to the post-trigeminal vein. The single pro-otic vein of the previous stage is replaced by several channels occupying the interval between ganglion V and the mesenchymal otic capsule: the smallest and most anterior of these crosses the root of ganglion V and joins the anterior end of the peri-trigeminal vein, the primitive superior petrosal sinus which lies, at its termination, between ganglion V and nerves III and IV.

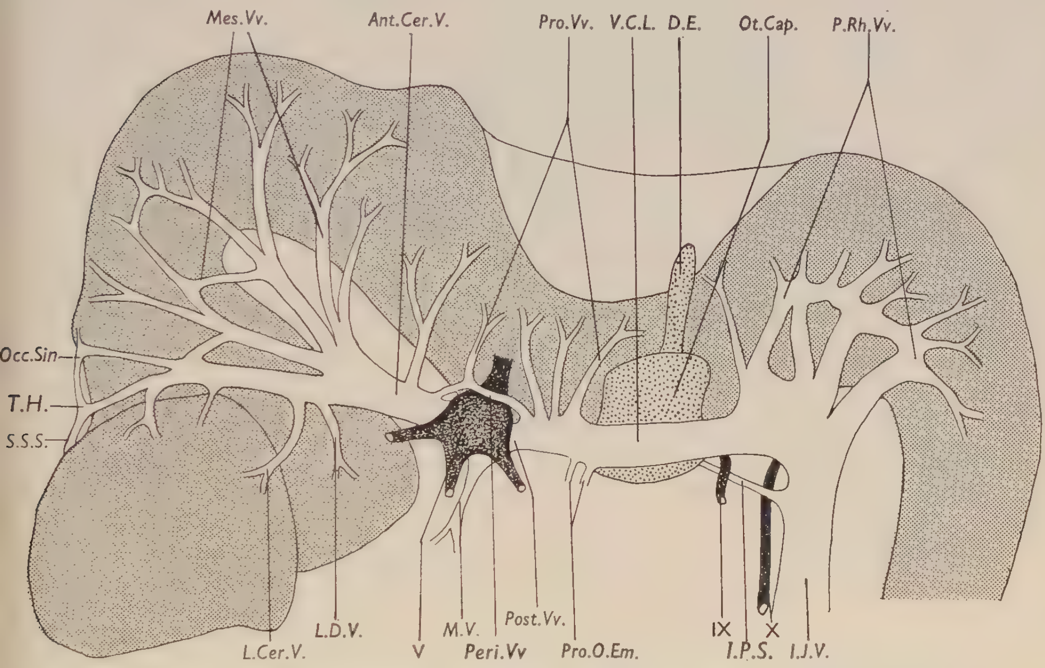

Fig. 2. Left lateral reconstruction of the head veins of a 14·0 mm. C.R. length embryo.

Similarly, the single posterior rhombencephalic vein is now replaced by several large channels joining the junction of the vena capitis lateralis and anterior cardinal vein. A small plexiform inferior petrosal sinus surrounds nerves IX and X and joins the anterior cardinal vein beyond the entry of the vena capitis lateralis, this junction marking the commencement of the internal jugular vein.

The vena capitis lateralis is in contact with the lateral surface of the mesenchymal otic capsule and receives only small tributaries into its lateral aspect. These connect it to the subectodermal venous plexus, the forerunner of the external jugular venous system in the otic region. The most prominent of these small veins, the pro-otic emissary veins, join the junction of vena capitis lateralis and the post-trigeminal vein opposite the entry of the main pro-otic veins.

Stage 3. 16·0–18·0 *mm. crown-rump length* (Fig. 3)

This stage is marked by the sudden appearance of a large supra-otic anastomosis connecting the pro-otic and posterior rhombencephalic veins. This new channel, together with the terminal part of the posterior rhombencephalic veins, forms the sigmoid sinus.

The anterior cerebral vein has a marked dorsal convexity, caused by the continued expansion of the cerebral hemisphere. Prior to its termination in the vena capitis medialis it receives a lateral cerebral and a lateral diencephalic vein. Close to its termination in the vena capitis medialis the maxillary vein receives the large superior ophthalmic vein and the maxillary vein is now recognized as the inferior

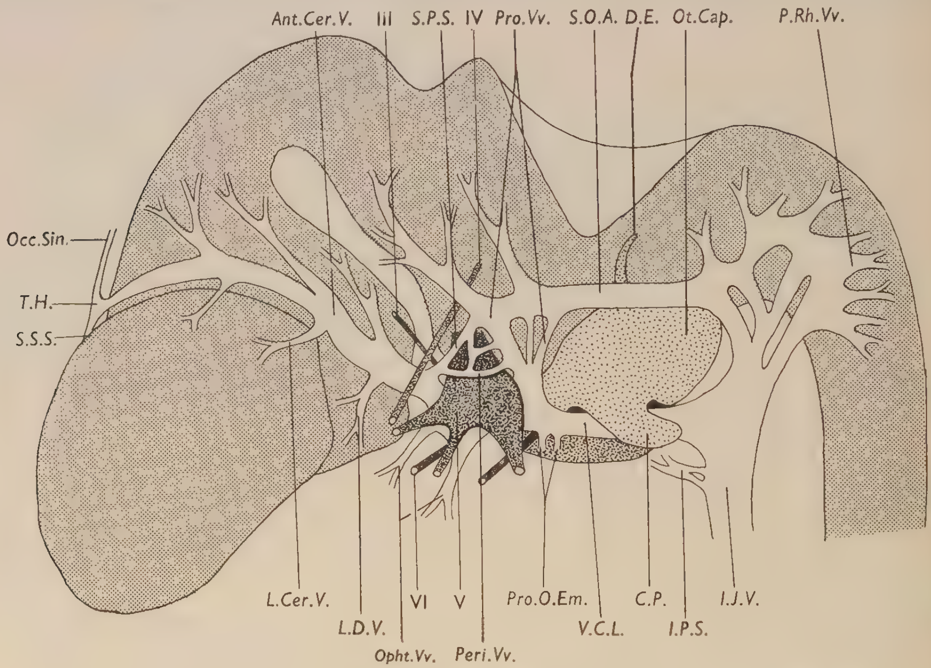

Fig. 3. Left lateral reconstruction of the head veins of 16·0–18·0 mm. C.R length embryos.

ophthalmic vein. The post-trigeminal vein still joins vena capitis medialis to the vena capitis lateralis. The latter vein runs caudalwards under the crista parotica (future tegmen tympani) lateral to the post-ganglionic part of nerve VII and medial to the stapedial artery. At the caudal end of the facial canal nerve VII turns laterally and forwards around the caudal extremity of the crista parotica, whereas the vena capitis lateralis continues caudally to enter the widely open occipito-capsular fissure. Here it joins the terminal part of the sigmoid sinus.

The post-trigeminal vein and its tributaries provide the key to the understanding of the subsequent history of the pro-otic veins, and it is therefore important to establish their relationships to the cranial nerves and chondrocranium. These veins are in the cavum epiptericum in close relation to ganglia V and VII. The geniculate ganglion is postero-medial to the junction of the post-trigeminal vein, the vena capitis lateralis and the pro-otic veins; the greater superficial petrosal nerve runs antero-laterally

below this junctional region. Two or three small, laterally directed veins connect this junctional region of the primary head vein to tributaries of the external jugular vein. They run lateralwards across the anterior aspect of the proximal end of Meckel's cartilage and are identical in position and connexions with the pro-otic emissary veins of the rat (the forerunners of the post-glenoid vein). They are in line with the termination of the pro-otic veins which now connect the commencement of the supra-otic anastomosis to the junction of the vena capitis lateralis and the post-trigeminal vein. The main pro-otic vein follows closely the caudal margin of ganglion V and is still a considerable distance anterior to the otic capsule. Smaller plexiform pro-otic veins, the diminutive superior petrosal sinus, now appears as an anteriorly directed branch of the main pro-otic vein joining either the peri-trigeminal vein or the termination of the anterior cerebral vein. The superior petrosal sinus lies in the cavum epiptericum close to the anterior layer of dura forming the tentorium cerebelli.

Two or three mastoid emissary veins pass through the upper part of the occipito-capsular fissure and connect the sigmoid sinus to occipital tributaries of the external jugular veins.

Stage 4. 21·0–23·0 *mm. crown-rump length*

(*a*) 21·0 *mm. embryo* (Fig. 4). The lateral sinus has been completed by the sudden appearance of an anastomotic channel, in line with the supra-otic anastomosis, connecting the anterior cerebral vein to the pro-otic veins. At the same time, the anterior cerebral vein disappears between the entry of the lateral cerebral vein and the vena capitis medialis. The completed lateral sinus is composed of: (1) the transverse sinus formed from the anterior cerebral vein from the torcular to the entry of the lateral cerebral vein (Fig. 4, *a–b*) and the new anastomosis between the anterior cerebral vein and the pro-otic veins (Fig. 4, *b–c*); and (2) the sigmoid sinus formed from the supra-otic anastomosis (Fig. 4, *c–d*) and the posterior rhombencephalic veins from the entry of the supra-otic anastomosis to their union with the internal jugular vein (Fig. 4, *d–e*).

The transverse sinus runs across the cavum epiptericum between the two layers of the tentorium cerebelli and, when the cavum epiptericum becomes reduced in size, will come to lie in the attached margin of the tentorium. During subsequent development the transverse sinus and the tentorium will rotate backwards through 180 degrees as a result of the continued caudal expansion of the cerebral hemispheres and the accentuation of the cerebral flexures.

The vena capitis medialis is greatly reduced in size, receiving the ophthalmic veins and the superior petrosal sinus, anteriorly, and the pituitary vein, medially. It continues behind the Vth ganglion as the post-trigeminal vein to join the greatly reduced vena capitis lateralis. Here the remnant of the primary head vein is joined by the somewhat more plexiform pro-otic veins which are still closer to the Vth ganglion than the otic capsule. A small pro-otic emissary vein joins the junction of the post-trigeminal vein and the vena capitis lateralis opposite the entry of the main pro-otic channels.

(*b*) 22·0 *mm. embryo*. The general pattern of veins is as in the previous embryo, but the middle part of the reduced vena capitis lateralis has disappeared in the region of the stapes, only its cranial and caudal ends remaining.

(*c*) 23·0 *mm. embryo* (Fig. 5). Vena capitis lateralis has completely disappeared and important changes have occurred in the pro-otic veins.

The transverse sinus now has a more vertical course and, when it reaches the antero-superior aspect of the otic capsule, it divides into two equal channels:

(1) The pro-otic vein or petro-squamous sinus running antero-ventrally in the angle between the parietal plate of cartilage and the otic capsule to enter the diminished interval between the last-named structure and the Vth ganglion. Here it lies in contact with the caudal margin of the ganglion, around which it bends medially

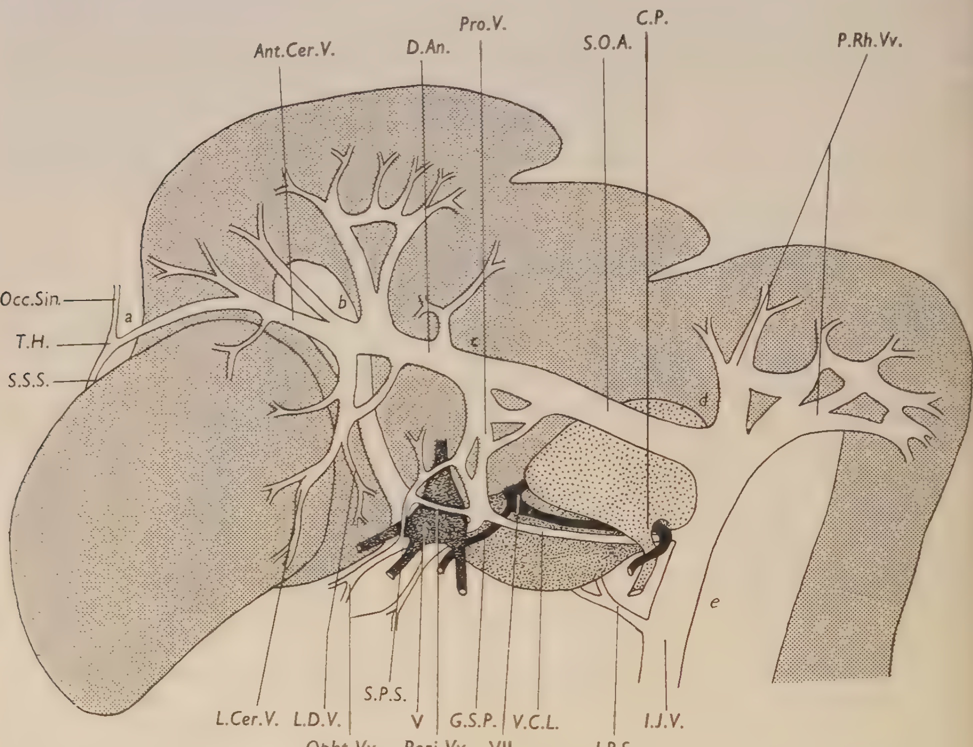

Fig. 4. Left lateral reconstruction of the head veins of a 20·0 mm. C.R. length embryo. *a–b–c* is the transverse sinus, *c–d–e* is the sigmoid sinus.

as the post-trigeminal vein terminating in the vena capitis medialis. As it bends around the Vth ganglion it receives two tiny pro-otic emissary veins. The greater superficial petrosal nerve runs forward below the termination of the petro-squamous sinus in the post-trigeminal vein. It is probable that the definitive petro-squamous sinus has formed from the more caudal of the earlier, multiple pro-otic veins and is thus an example of spontaneous migration of a venous channel (Streeter, 1915, 1918). It gives off a small anteriorly directed branch which crosses the superior surface of the Vth ganglion to join the anterior extremity of the vena capitis medialis; the superior petrosal sinus.

(2) The sigmoid sinus which follows the superior and posterior surfaces of the otic capsule, lateral to the ductus endolymphaticus, first in the parieto-capsular

angle and then in the occipito-capsular angle. It leaves the medial and inferior end of the occipito-capsular fissure which has now been cut off from the superior part of the fissure by the jugular process of the ex-occipital to form the jugular foramen. The superior part of the occipito-capsular fissure is reduced to two foramina, lying at the superior aspect of the otic capsule, and transmitting large mastoid emissary veins. Outside the jugular foramen the inferior petrosal sinus joins the internal jugular vein.

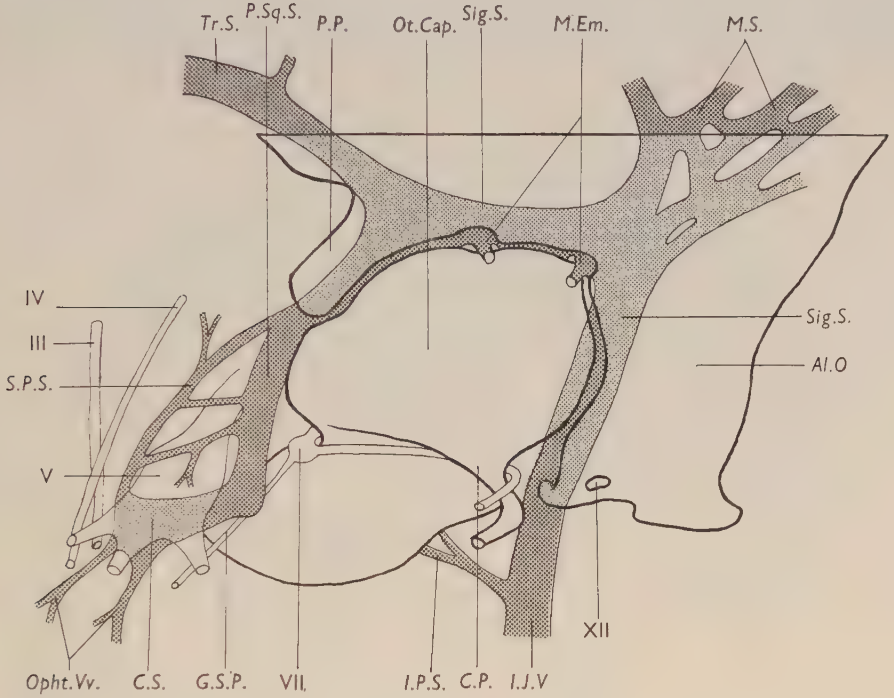

Fig. 5. Left lateral reconstruction of the otic region of the chondrocranium and associated dural venous sinuses and cranial nerves. 23·0 mm. C.R. length embryo.

This embryo is unusual in so far as the terminal part of the anterior cerebral vein is still present, albeit reduced in size. It is recognized because, as it enters the anterior end of the vena capitis medialis, it passes between nerves III and IV. It follows the caudal margin of the cartilaginous ala orbitalis of the sphenoid and, were it to have persisted into adult life, would have become the inconstant sinus alae parvae.

The diminished vena capitis medialis lies in the depths of the reduced cavum epiptericum between the Vth ganglion and the pituitary. The internal carotid artery and the VIth nerve run forwards below it and the nerves III and IV are anterior. These relationships establish, without any doubt, that it is the forerunner of the cavernous sinus although it appears as a wide, blood-filled space with no evidence of trabeculation and has not yet expanded so as to include the surrounding structures in its wall. The middle meningeal artery ascends in the lateral part of the cavum epiptericum lying upon the lateral surface of the petro-squamous sinus. Its anterior

and posterior branches are accompanied by venae comitantes which enter the petro-squamous sinus.

B. *The foetal period, 9–25 weeks*

Stage 5. 30·0–47 *mm. crown-rump length* (Fig. 6)

The main feature of this stage is the petro-squamous sinus which is almost as large as the sigmoid sinus. It curls around the caudal margin of the Vth ganglion to enter the caudal end of the cavernous sinus. About half-way along its length it gives off a small, often plexiform, anteriorly directed branch which crosses over the Vth ganglion to enter the cranial end of the cavernous sinus; the superior petrosal sinus.

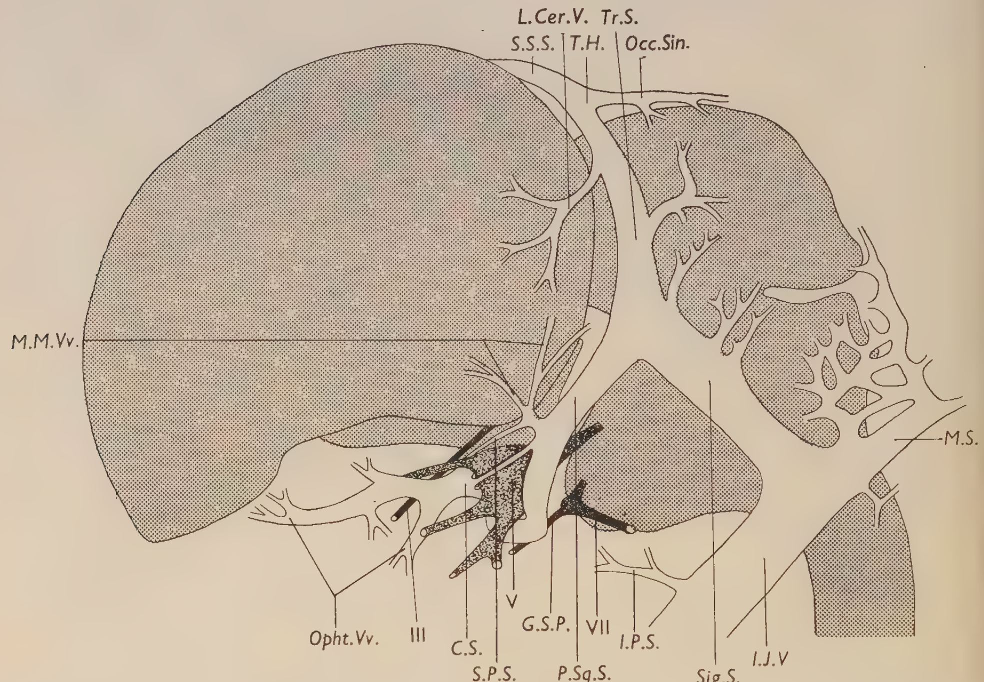

Fig. 6. Left lateral reconstruction of the dural venous sinuses of a 45·0 mm. C.R. length foetus.

The venae comitantes of the anterior and posterior branches of the middle meningeal artery join the petro-squamous sinus at the origin of the superior petrosal sinus. As yet there are no venae comitantes with the stem of the middle meningeal artery. The pro-otic emissary veins have disappeared, although they may occasionally persist into adult life. The arrangement of the remaining dural venous sinuses shows little change.

Stage 6. 60·0–175·0 *mm. crown-rump length* (Fig. 7)

In the older foetuses of this stage the tentorium cerebelli and contained transverse sinus have become rotated almost into their adult position. In addition, there has been considerable expansion of the temporal lobe of the cerebrum and a corresponding excavation of the middle cranial fossa. In the 99·0 mm. foetus (Fig. 7) the

petro-squamous sinus is beginning to disappear and, in order of size, the three divisions of the transverse sinus are:

(1) The sigmoid sinus, by far the largest, running in the sigmoid groove to the jugular foramen. It gives off two large mastoid emissary veins passing out of the skull through a pair of superior occipito-capsular foramina. These are now situated posterior to the otic capsule which has been tilted backwards as a result of the excavation of the middle cranial fossa (cf. Figs. 5 and 7).

(2) The petro-squamous sinus is now considerably reduced, particularly at its commencement. It runs along the antero-lateral face of the otic capsule, in the parieto-capsular angle, then across the medial part of the middle cranial fossa to end

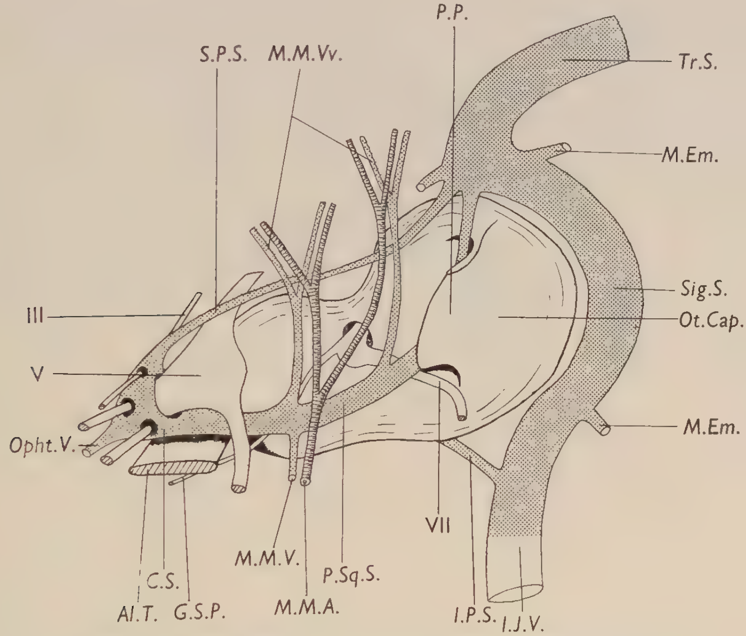

Fig. 7. Left lateral reconstruction of the otic capsule and associated dural venous sinuses and cranial nerves. 99·0 mm. C.R. length foetus.

in the cavernous sinus deep to the Vth ganglion. The venae comitantes accompanying the anterior and posterior branches of the middle meningeal artery now join the petro-squamous sinus separately and some distance apart. The main reduction in the petro-squamous sinus occurs prior to the entry of the posterior group of middle meningeal veins. Via the terminal part of the petro-squamous sinus the middle meningeal veins drain into the cavernous sinus but a new drainage route is now apparent. Around the stem of the middle meningeal artery there are venae comitantes connecting the petro-squamous sinus to the pterygoid venous plexus. They leave the skull, with the artery, in the fissure between the ala temporalis and the otic capsule. Thus is laid down the dual termination of the adult middle meningeal veins. In the older foetuses a large anastomotic channel connects the anterior and posterior middle meningeal veins. It was seen first in a foetus of 85·0 mm. C.R. length

and lies parallel to, but considerably lateral of, the petro-squamous sinus from which it is separated by the cranial surface of the tegmen tympani (Fig. 8).

(3) The superior petrosal sinus is now only slightly smaller than the commencement of the petro-squamous sinus and runs along the upper margin of the otic capsule and then above the Vth ganglion in the roof of the cavum Meckelii. As it joins the cavernous sinus it lies between the Vth ganglion below, and the IIIrd and IVth nerves above.

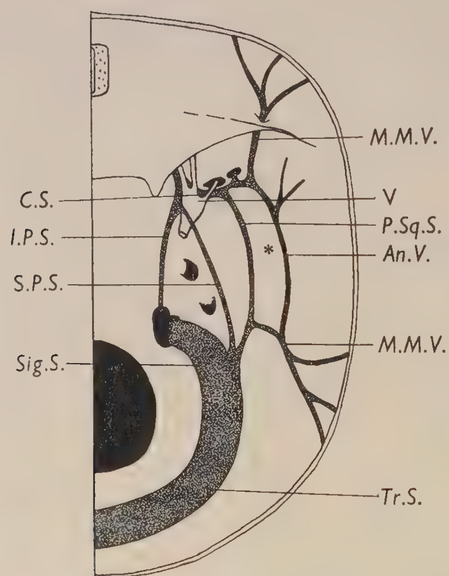

Fig. 8. Middle meningeal veins and certain dural venous sinuses of an 85·0 mm. C.R. length foetus. * = cranial surface of the tegmen tympani.

The wall of the cavernous sinus is now invaginated by the surrounding structures: internal carotid artery, V*a*, V*b*, and the IIIrd, IVth and VIth nerves. There are no trabeculae and in no way does the sinus resemble cavernous tissue. Each sinus lies, with the surrounding artery and nerves, in the depths of the greatly reduced cavum epiptericum.

C. *Adult period*

(1) *The middle meningeal veins and petro-squamous sinus*

The vascular grooves in thirty adult skulls revealed patterns in accord with the arrangement of the foetal middle meningeal veins. Fig. 9A shows a skull with a well-marked groove for the petro-squamous sinus receiving widely separated grooves for the anterior and posterior middle meningeal veins and is to be compared with the arrangement seen in a 99·0 mm. C.R. length foetus (Fig. 7). The petro-squamous sinus now terminates in the anterior middle meningeal vein and so joins the pterygoid venous plexus via the foramen spinosum. Not infrequently, however, its primitive connexion with the cavernous sinus may persist as a channel draining the anterior middle meningeal vein into the cavernous sinus. Fig. 9B is essentially the

same but with the addition of a groove for the anastomotic vein between the anterior and posterior middle meningeal veins, such as was seen in an 85·0 mm. C.R. length foetus (Fig. 8). One example of each of the above types was found. Fig. 9C shows a pattern in which the terminal part of the posterior middle meningeal vein has disappeared but a small petro-squamous sinus persists. Two such examples were found and in one of them a small post-glenoid foramen opened internally into the terminal groove, common to the middle meningeal veins and the petro-squamous sinus. The remaining twenty-six skulls showed the pattern usually depicted (Fig. 9D), and in two of them a small post-glenoid foramen opened into the groove for the common stem of the middle meningeal veins.

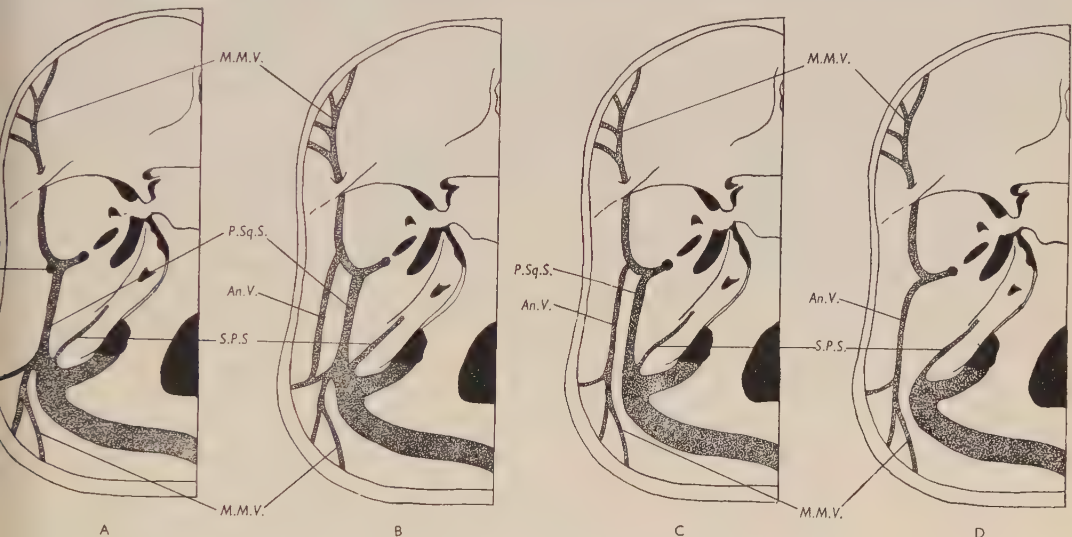

Fig. 9. Patterns of vascular grooves found in adult human skulls.

(2) *The cavernous sinus*

Dissection of sixteen cavernous sinuses, from individuals aged between 8 and 80 years, revealed very few trabeculae. These were situated mostly around the periphery of the sinus, close to the entry of tributaries. Small Pacchionian bodies projected into the cavity of the sinus.

DISCUSSION

In general, it may be said that the morphological interpretation of the developing human dural venous sinuses described above is in accord with that given by Markowski (1911, 1922) and not that of Streeter (1915, 1918). The functional interpretation put forward by Streeter, i.e. the manner whereby the developing head veins adjust themselves to growth changes of the brain and cranium was amply confirmed.

(1) *Vena capitis lateralis*

The human vena capitis lateralis is identical in position and relationships with that found in other mammalian embryos (guinea-pig, Salzer, 1895; monotremes, Hochstetter, 1896; bats, Grosser, 1901; and rat, Butler, 1953). It is extra-dural

and therefore morphologically extra-cranial in the sense defined by Sutton (1888). It does, however, emerge from the chondrocranium at the posterior end of the spheno-parietal fontanelle before running caudalwards, with the VIIth nerve, beneath the crista parotica. The otic region of the chondrocranium becomes covered by the squamous temporal and the tympanic ring except the extreme anterior tip of the crista parotica, which obtrudes into the Glaserian fissure, and the pars mastoidea of the petrous temporal. Therefore, should this vein persist into adult life it would become embedded in the adult cranial wall, running in the facial nerve canal and emerging via the primitive stylo-mastoid foramen to lie upon the outer surface of the pars mastoidea. It would then join the terminal part of the sigmoid sinus as it emerges from the jugular foramen to form the commencement of the internal jugular vein. Such an arrangement is found in adult monotremes (Hochstetter, 1896) where vena capitis lateralis does persist into adult life, but these animals have no post-glenoid vein.

It was shown in the rat (Butler, 1953) that the post-glenoid vein is developed from small pro-otic emissary veins which connect vena capitis lateralis, at the point where it enters the facial canal, to temporal tributaries of the external jugular vein. These small veins run lateralwards across the anterior surface of the proximal end of Meckel's cartilage. They enter the vena capitis lateralis directly opposite the termination of the petro-squamous sinus, and it is only this small junctional region of vena capitis lateralis which enters into the formation of the post-glenoid vein.

The oft-repeated statement that the vena capitis lateralis leaves the skull between nerves V and VII (Mall, 1905; Hochstetter, 1906; Evans, 1912) is only true if by skull is meant chondrocranium. The post-trigeminal part of the primary head vein, linking the vena capitis medialis to the vena capitis lateralis, does run lateralwards between these two nerves to leave the posterior end of the spheno-parietal fontanelle as the commencement of the vena capitis lateralis. The vena capitis lateralis cannot leave by the post-glenoid foramen formed between the squamous temporal and the tympanic ring because it has disappeared before these bones are laid down. It is the pro-otic emissary veins, running lateralwards from vena capitis lateralis, which come to lie between these bones.

(2) *Petro-squamous sinus*

The main channel which develops in the pro-otic group of veins lies, at first, along the caudal border of the Vth ganglion some distance anterior to the otic capsule. It gradually moves caudally, probably by spontaneous migration, so as to lie on the anterior surface of the otic capsule in the parieto-capsular angle (Fig. 10 A). It never lies upon the superior margin of the otic capsule and is close to the inner surface of Dürer's membrane and is in contact with the middle meningeal artery, laterally. At its termination in the junction of vena capitis lateralis and the post-trigeminal vein it lies between the Vth and VIIth ganglia. The pro-otic emissary veins join the lateral aspect of this junctional region and the greater superficial petrosal nerve runs forwards below it. Dorsally, the main pro-otic channel joins the junction of the transverse and sigmoid sinuses. In view of these relationships this channel must be the petro-squamous sinus. Despite the fact that Streeter only indicates the membranous labyrinth and the Vth ganglion in his reconstructions, the channel that

he calls superior petrosal sinus can be none other than the pro-otic vein of Markowski (1911, 1922) which, as has been explained, is the petro-squamous sinus.

During early foetal life (30·0–47·0 mm. C.R. length) the petro-squamous sinus is almost as large as the sigmoid sinus and it receives the middle meningeal veins (Fig. 6). In foetuses of 60·0 mm. C.R. length and more, it usually declines in size but frequently persists into post-natal life. It receives scant attention in most modern text-books of anatomy, being generally regarded as an occasional variant. Bell (1829) reckoned it a normal feature of adult anatomy, calling it the anterior petrosal sinus. Knott (1882) found a petro-squamous sinus in twenty-six out of forty-four adult cadavers (seven bilateral, nineteen unilateral). Cheatle (1889) stated: '... it

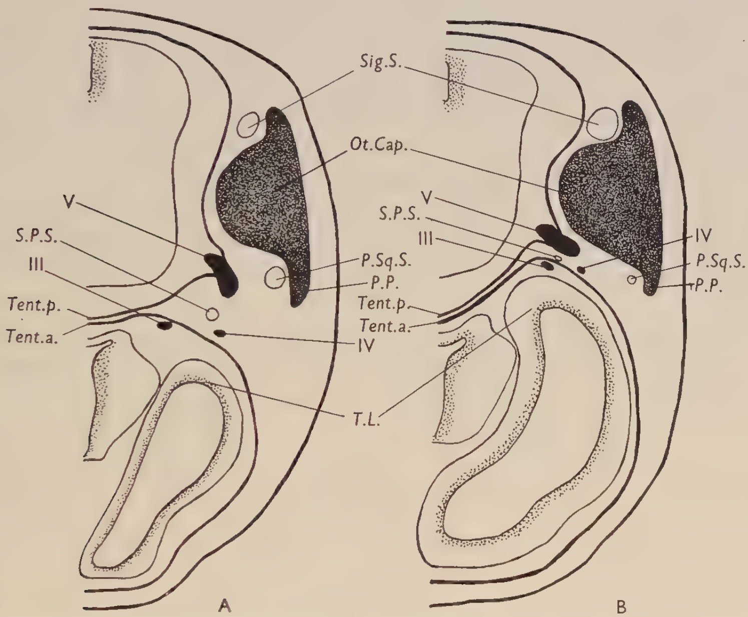

Fig. 10. Diagrams to illustrate the reduction of the cavum epiptericum by the expansion of the temporal lobe of the cerebrum. Constructed from sections: A, 23·0 mm. C.R. length embryo; B, 60·0 mm. C.R. length foetus.

is the rule rather than the exception for remains of the sinus to be present in some form or other all through life. It is usually well marked in infancy and childhood and anteriorly joins the middle meningeal veins.' Only four of the thirty skulls examined showed a petro-squamous groove, but a sinus may be present without a groove in the bone. The large size of the petro-squamous sinus in early foetal life is in accord with its prevalence in adults and it should be regarded as a usually occurring sinus and not an occasional variant.

(3) *Post-glenoid vein*

A rudimentary post-glenoid foramen occurs in slightly under 1·0% of adult human skulls (Cheatle, 1889; Boyd, 1930), and this is in accord with the small size and transitory appearance of the pro-otic emissary veins of the human foetus.

(4) *Superior petrosal sinus*

In adult man this sinus leaves the postero-superior aspect of the cavernous sinus and runs caudally below the nerves III and IV and bridges over the superior aspect of the Vth ganglion in the roof of the cavum Meckelii. The main pro-otic channel, which Streeter called the superior petrosal sinus, does not have these nervous relationships, but they are fulfilled by the smaller and most anterior group of pro-otic veins. These run forwards across the lateral face of the Vth ganglion and join either the peri-trigeminal vein or the vena capitis medialis (the cavernous sinus). At their termination they lie between the nerves III and IV anteriorly, and the Vth ganglion posteriorly.

When first developed the anterior layer of the tentorial fold is reflected on to the inner surface of the orbital cartilage, well anterior to the otic capsule, and the cavum epiptericum is wide in an antero-posterior direction. The IIIrd and IVth nerves and the superior petrosal sinus are close to the anterior dural fold and well in front of the otic capsule, from which they are separated by the Vth ganglion (Fig. 10A). The posterior tentorial fold runs along the superior border of the otic capsule and then sweeps on to the posterior surface of the otic capsule. As the temporal lobe of the cerebral hemisphere expands and excavates the middle cranial fossa it presses the anterior tentorial layer backwards, together with the IIIrd and IVth nerves, the Vth ganglion and the superior petrosal sinus, towards the otic capsule. The cavum epiptericum is thereby reduced in size and the line of attachment of the anterior tentorial fold comes to lie alongside that of the posterior layer, along the superior border of the otic capsule (Fig. 10B). Thus the nerves and the sinus attain their adult positions and the superior petrosal sinus runs above the Vth ganglion and below the nerves III and IV (Figs. 7, 10B). The expanded cavernous sinus now protrudes anteriorly and superiorly of the Vth ganglion and here receives the superior petrosal sinus.

(5) *Cavernous sinus*

This is developed from the vena capitis medialis and, in late foetal life, it expands to envelop the internal carotid artery and nerves V*a*, V*b*, III, IV and VI. No trabeculae were present in the embryonic or foetal material and very few appeared in adult specimens. There was no evidence that the sinus was formed by the coalescence of a capillary plexus such as gives rise to the superior sagittal sinus and its contained chordae Willisii. Such trabeculae as were found occurred at the periphery of the sinus, close to the entry of tributaries, and it is suggested that they may have been formed by the taking up of plexiform tributaries during the expansion of the cavernous sinus. (No trabeculae were found in the cavernous sinus of adult rats, and Eyster (1944) reported a similar absence of trabeculae in the cavernous sinus of the macaque monkey.)

Sections through a distended adult cavernous sinus showed no hint of cavernous tissue but, when the sinus is collapsed, as is usual in dissecting room cadavers, its cavity is encroached upon by the nerves and Pacchionian bodies in its wall. This readily gives a spurious resemblance to cavernous tissue.

(6) *Middle meningeal veins*

The variations in these veins need little comment and are intimately related to the petro-squamous sinus. The mode of middle meningeal vein development offers a ready explanation for their dual termination in the cavernous sinus and the pterygoid venous plexus.

SUMMARY

1. The development of certain human dural venous sinuses has been investigated in a series of twenty embryos and foetuses ranging from 7·5 to 175·0 mm. C.R. length.

2. The pro-otic veins form the large petro-squamous sinus and the much smaller superior petrosal sinus. In foetuses of 40·0–45·0 mm. C.R. length the former sinus is as large as the sigmoid sinus. Thereafter it declines in size but frequently persists into post-natal life. It receives the middle meningeal veins.

3. Minute pro-otic emissary veins, forerunners of the post-glenoid vein, are found in embryos up to 23·0 mm. C.R. length. Thereafter they usually disappear, though a post-glenoid foramen is found in a little under 1·0 % of adult human skulls.

4. The cavernous sinus is the expanded remnant of the vena capitis medialis. Very few trabeculae occur in the adult sinus, which bears no resemblance to cavernous tissue.

My thanks are due to Prof. J. D. Boyd, Anatomy School, Cambridge, for the generous way in which he placed his unique collection of sectioned human embryos and foetuses at my disposal and to Dr A. M. Barrett, Pathologist to the University of Cambridge, for specimens of adult cavernous sinuses.

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ABBREVIATIONS

Ant.Cer.V.	anterior cerebral vein	*Post V.*	post-trigeminal vein
A.C.V.	anterior cardinal vein	*Peri V.*	peri-trigeminal vein
Al.O.	ala occipitalis	*P.P.*	parietal plate of cartilage
Al.T.	ala temporalis	*P.Sq.S.*	petro-squamous sinus
An.V.	new channel between anterior and posterior middle meningeal veins	*P.G.F.*	post-glenoid foramen
		P.Rh.V.(Vv.)	posterior rhombencephalic vein(s)
C.P.	crista parotica	*S.S.S.*	superior sagittal sinus
C.S.	cavernous sinus	*Sig.S.*	sigmoid sinus
D.An.	dorsal anastomosis	*S.P.S.*	superior petrosal sinus
D.E.	ductus endolymphaticus	*S.O.A.*	supra-otic anastomosis
G.S.P.	greater superficial petrosal nerve	*T.H.*	torcular Herophili
I.J.V.	internal jugular vein	*Tent.a.*	anterior layer of the tentorium cerebelli
I.P.S.	inferior petrosal sinus		
L.Cer.V.	lateral cerebral vein	*Tent.p.*	posterior layer of the tentorium cerebelli
L.D.V.	lateral diencephalic vein		
M.Em.	mastoid emissary vein(s)	*Tr.S.*	transverse sinus
M.M.A.	middle meningeal artery	*T.L.*	temporal lobe of cerebrum
M.M.V.(Vv.)	middle meningeal vein(s)	*V.C.M.*	vena capitis medialis
M.S.	marginal sinus	*V.C.L.*	vena capitis lateralis
M.V.	maxillary vein	III	third nerve
Mes.Vv.	mesencephalic veins	IV	fourth nerve
Ot.V.	otic vesicle	V	trigeminal ganglion
Ot.Cap.	otic capsule	VI	sixth nerve
Occ.Sin	occipital sinus	VII	seventh nerve
Opht.V.(Vv.)	ophthalmic vein(s)	IX	ninth nerve
Pro.V.(Vv.)	pro-otic vein(s)	X	tenth nerve
Pro.O.Em.	pro-otic emissary veins	XII	hypoglossal foramen

THE EFFECT OF FIXATION ON THE AMELOBLAST LAYER OF THE ENAMEL ORGAN IN THE RAT

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It was found that the appearance of the ameloblast layer of the developing tooth varied considerably with a number of different fixatives. It was decided to investigate this further. Moreover, it was thought that some light might be shed on the appearance of the two forms of cell which are seen in the ameloblast layer when 40 % formalin (16 % formaldehyde), chilled to 3–4° C., is used as the fixative (Symons, 1955); for with the more common fixatives all the cells of this layer appear similar.

The effect of a range of fixatives was studied on material which was cut tangentially to the convex surface of the tooth, so that the ameloblast layer was seen in transverse section. This gave the most favourable conditions for examining individual cells of the layer.

MATERIAL AND METHODS

The lower incisor teeth of 2-day-old rats were investigated. The material was fixed in 10 % formalin (4 % formaldehyde), 40 % chilled formalin (16 % formaldehyde), Bouin, Zenker, Helly, or absolute alcohol for 36 hr. The subsequent treatment in each specimen was the same, viz. dehydration in ethyl alcohol, clearing in benzene and embedding in paraffin wax; except that the formalin and absolute alcohol-fixed material was decalcified in 4 % nitric acid. This decalcification did not appear to alter the form of the cells in the ameloblast layer. The natural acidity of the other fixatives used was found to be sufficient for decalcification.

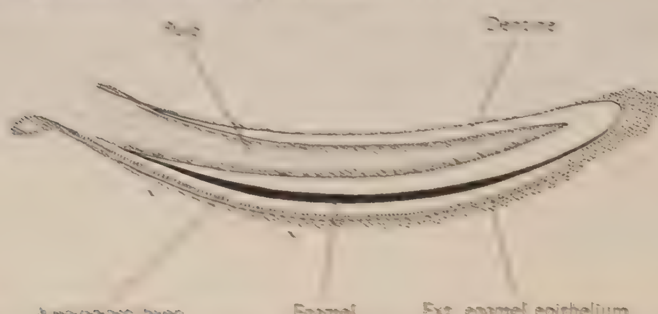
In some cases the formalin was neutralized with magnesium carbonate (pH 7·6); in others with calcium carbonate (pH 6·4). Neutralization with calcium carbonate appeared to give much better results.

The material was sectioned serially at 4μ thickness, and all sections were stained with Mayer's haemalum and eosin.

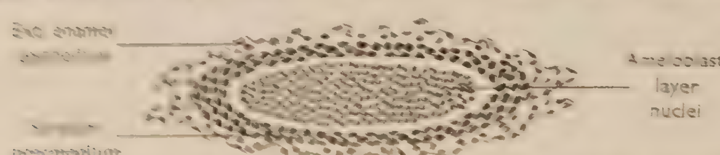
Orientation. The lower incisor of the 2-day-old rats was sectioned tangentially to the convexity of the anterior surface in the region of early enamel matrix formation (Text-fig. 1). The outlines of the sections were elliptical and became progressively elongated as the sections went deeper. As the sections were followed, the various layers of the developing tooth appeared in succession in the centre of the ellipses (Text-fig. 2). Each new tissue displaced the others outward so that in deeper sections there was a series of concentric zones; first the outer cells of the reduced enamel organ, then the stratum intermedium, the ameloblasts, the developing enamel, the developing dentine and finally the pulp tissue. The most informative sections were those in which the ameloblasts occupied the centre of the elliptical field. In a few blocks sectioned nearer the basal formative part of the incisor, the internal enamel epithelium replaced the ameloblast layer.

OBSERVATIONS

Nuclei. In sections through the ameloblast layer from material fixed in 40% salted formalin, two kinds of nuclei were seen. The most numerous form was large, oval or rounded in outline, and stained blue. The second was smaller and less numerous. These were angular, had flattened sides, and stained a deep purplish colour (Pl. 1, fig. 1). Their cytoplasmic zones around the smaller nuclei were connected to each other by delicate processes, very difficult to photograph. In general, the two nuclear forms were easily distinguished. Sections cut through the internal enamel epithelium from similarly fixed material also showed the two kinds of nuclei, but here they were present in about equal numbers (Pl. 1, fig. 8).



Text-fig. 1. Diagrammatic representation of a sagittal section of a lower incisor of a 2-day-old rat. The area between the marks X - - - X indicates the region where sections tangential to the developing tooth were cut, in order to obtain transverse sections of the ameloblast layer.



Text-fig. 2. Diagram of a tangential section of the lower incisor of a 2-day-old rat, which shows the various layers of the enamel organ.

In sections from material fixed in 10% formalin it was much more difficult to distinguish the two kinds of nuclei. In fact without the evidence from the 40% formalin-fixed material it is doubtful whether the two kinds of nuclei would ever have been recognized. On careful examination of material fixed in 10% formalin it could be seen that some nuclei were a little less rounded than others and stained somewhat purplish.

In material fixed in Bouin, the nuclei were all very similar in form; they were rounded, with here and there a suggestion of crenation (Pl. 1, fig. 2). The circumnuclear cytoplasm appeared shrunken. Sections of Bouin-fixed material from the internal enamel epithelium were essentially similar.

In material fixed in Zenker, also, the nuclei in the ameloblast layer appeared similar in form. They were, however, so densely stained that no nuclear detail could be made out. Moreover, they appeared rather distorted. As a result of this and the bunching together of nuclei that occurred in places, it was often difficult to determine the exact outline of each nucleus (Pl. 1, fig. 3). Some irregular inter-nuclear

material was seen. Sections of the internal enamel epithelium gave a similar appearance.

In sections of the ameloblast layer from material fixed in Helly, the nuclei were all alike in appearance. They were large and closely packed. It would seem that at some time in the course of histological preparation the nuclei may have been tightly pressed together, for many had flattened sides (Pl. 1, fig. 4). A similar appearance of the nuclei was seen in sections of the internal enamel epithelium.

In material fixed in absolute alcohol, the nuclei of the ameloblast layer were mainly large and round, but amongst them were some which were much smaller and more deeply stained (Pl. 1, fig. 5). That these latter were not sections through the upper or lower poles of certain of the large nuclei was determined by careful focusing at different levels in the series. Absolute alcohol-fixed material from the internal enamel epithelium also showed the two kinds of nuclei, but the smaller nuclei were much more numerous than in the ameloblast layer (Pl. 1, fig. 6).

Distal zone of the cells. Sections of material fixed in 40 % chilled formalin and cut through the distal zones of the cells of the ameloblast layer showed two differently stained cytoplasmic elements (Pl. 1, fig. 7). One, staining a deep purplish pink, appeared to form a network surrounding a much lighter bluish pink element. The pattern found in sections of the cytoplasm of the internal enamel epithelium was very similar except that the contrast between the two cytoplasmic elements was even more marked, the purplish pink network being very prominent indeed (Pl. 1, fig. 8). When the cells of the ameloblast layer were traced through the serial sections to the surface of the developing enamel, the purplish pink network became continuous with the 'honeycomb' of the developing enamel matrix.

Sections from similar material fixed in Bouin gave a vastly different picture. The cytoplasm was broken up into triangular or polygonal areas, which were connected to each other by fine processes, and formed a fairly regular pattern (Pl. 1, fig. 9). On first inspection, the cytoplasmic areas appeared to be relatively homogeneous, but in certain places darker streaks, frequently close to one side, could be seen crossing them (Pl. 1, fig. 10). It was not always easy to define the boundaries of the cytoplasmic areas, especially when they were closely packed. Near the surface of the developing enamel the cytoplasmic pattern became disorderly, and numerous circular gaps appeared between the cytoplasmic areas (Pl. 1, fig. 9). It was not possible to determine the relationship of the cytoplasm to the developing enamel matrix in Bouin-fixed material.

The appearances seen in material fixed in Zenker, Helly and absolute alcohol were very similar to those described for the Bouin-fixed material. There were a few minor differences. In the Zenker-fixed material the boundaries of the triangular and polygonal areas of the cytoplasm were somewhat less distinct. This indefiniteness was even more marked in the absolute alcohol-fixed material. With alcoholic fixation the matrix of the developing enamel disintegrated during decalcification, so that the cells of the ameloblast layer became detached and disarranged. In the Helly-fixed material the intervals between the triangular and polygonal areas of cytoplasm were very narrow throughout the ameloblast layer and, towards the developing enamel, no gaps resembling those in the Bouin, Zenker and absolute alcohol-fixed material were found.

DISCUSSION

Perhaps the most remarkable feature of the results is the variation in the appearance of the ameloblast layer produced by each of the fixatives used; and particularly of the nuclei. In material fixed in 40 % chilled formalin, two nuclear forms were seen. With 10 % formalin the two nuclear forms were much less easy to distinguish than after 40 % chilled formalin.

Material fixed in absolute alcohol again showed two forms of nuclei in transverse sections, although in longitudinal sections the darker nuclei could not be seen, possibly because they were hidden by the very much larger nuclei, or through being mistaken for nucleolar material. The alcohol-fixed material showed no such cytoplasmic differentiation as was found in specimens after 40 % chilled formalin. Like these specimens the alcohol-fixed material showed a larger proportion of the small dark nuclei in the internal enamel epithelium than in the ameloblast layer.

In material fixed in Bouin or Zenker, only one nuclear type seemed to be present, though it is possible in the case of the Zenker-fixed material that the nuclear distortion and density of staining could obscure any differences that might have existed.

It has been pointed out that the nuclei in Helly-fixed material were closely packed and would appear to have been at some stage possibly even more closely pressed, suggesting that this fixative produces a considerable initial swelling followed later by some shrinkage. It is worth noting that Baker (1951, pp. 30–31) points out that the chief components of Helly, viz. potassium dichromate, mercuric chloride and formalin, all produce an initial increase in volume. The initial swelling produced by Helly-fixation might obliterate any morphological differences between the nuclei of the ameloblast layer such as are revealed by 40 % formalin.

It is interesting to find that the differences in nuclear appearance bear out exactly what Baker (1951, p. 91) says concerning the effects of Zenker and Helly. Baker points out that, though the two fixatives are so similar in their formulae, they are profoundly different in action. In Zenker, due to the addition of glacial acetic acid, the pH is as low as 2·3 and, as a result, the dichromate acts as chromic acid. Thus in Zenker there are three strong protein precipitants present, viz. chromic acid, mercuric chloride, and acetic acid. In Helly, however, the addition of formalin instead of acetic acid keeps the pH at 4·7, and the dichromate does not act as a protein precipitant. The striking differences in the effect of these two fixatives are shown in Pl. 1, figs. 3 and 4.

It is thought that the appearance of the distal cytoplasmic zone of the cells of the ameloblast layer, obtained after 40 % chilled formalin, are likely to be nearer the condition in life than that seen after other fixatives. There is no break in the continuity of the cytoplasmic elements, whereas with the other fixatives the cytoplasm is distorted into triangular or polygonal areas separated by shrinkage spaces. Moreover, at the region of junction between the cells and the developing enamel there appeared to be greater order and clarity in the formalin-fixed than in the other material.

It is difficult to relate the appearance of this region obtained after 40 % chilled formalin to that seen after other fixatives. If the formalin-fixed material gives the truest picture, it must be supposed that the triangular and polygonal areas of

cytoplasm seen after the other fixatives are somehow produced from the continuous cytoplasmic pattern shown by the formalin-fixed material.

The two nuclear and cytoplasmic elements seen in transverse sections of the ameloblast layer after fixation in 40% chilled formalin are complementary to the appearances seen in longitudinal sections after this fixative (Symons, 1955); and suggest that two distinct cell forms may be present in the ameloblast layer. The term, kionoblast, was employed by Saunders, Nuckolls and Frisbie (1942) to distinguish the dark-staining cell from the lighter-staining ameloblast.

It has been shown that the darker cytoplasmic bodies appear to form a network around the ameloblasts, and that the former connect directly with the 'honeycomb' matrix around the developing enamel prisms. This agrees with the suggestion already made (Symons, 1955) that the kionoblasts may manufacture the interprismatic substance of the enamel (assuming that the 'honeycomb' matrix represents the developing interprismatic substance; for the relationship of this matrix to the prism sheaths is very uncertain). Jasswoin (1924) described and figured 'narrow and flattened cells' among the typical ameloblasts which he, however, considered were immature forms. He thought that these cells, before their differentiation into fully mature ameloblasts, might be responsible for the production of the 'ectoplasmic substance' by which he appeared to mean the interprismatic substance of the enamel.

The sensitivity of the ameloblast layer to differences in fixation appears to be greater than that of most other tissues. Other tissues seen in the sections, such as muscle, bone, cartilage and fibrous tissue, only exhibited minor differences between the various fixatives. Even the cells of the stratum intermedium appeared much the same after all the fixatives used. The explanation may possibly be that the cells of the ameloblast layer contain a high percentage of water or some labile material which is lost with most fixatives leading to distortion of the cells.

SUMMARY

1. The lower incisors of 2-day-old rats have been fixed with 10% formalin, 40% chilled formalin, Bouin, Zenker, Helly or absolute alcohol, followed in each case by routine dehydration, clearing and embedding in paraffin wax.

2. Very marked differences are produced in the appearance of the ameloblast layer by this range of fixatives; these differences are best appreciated in transverse sections of the ameloblast layer.

3. With 40% chilled formalin-fixation two forms of cells are seen in the ameloblast layer; after the other fixatives all the cellular elements of the ameloblast layer seem to be identical. With absolute alcohol-fixation, however, two kinds of nuclei are found.

4. It is concluded that 40% chilled formalin gives an appearance closer to what obtains in life since with it there is no break in the continuity of the cytoplasm, and a clear and orderly connexion between the cells and the matrix of the developing enamel is found; whereas with the other fixatives the cellular pattern seems distorted and no clear relation between cells and enamel matrix can be defined.

I wish to thank Prof. A. D. Hitchin for his interest in this work. I am also indebted to Prof. J. J. Pritchard for his helpful criticism of the manuscript.

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EXPLANATION OF PLATE

All the figures are of material from the same litter of rats.

Fig. 1. Transverse section of the nuclei of ameloblast layer from lower incisor of 2-day-old rat, fixed in 40 % chilled formalin. Two kinds of nuclei are seen, one large, oval or roundish and lightly stained, and the other smaller, angular, with flattened sides, and deeply stained. *s*, small dark nucleus. H. & E. ×1200.

Fig. 2. Transverse section of the nuclei of ameloblast layer from lower incisor of 2-day-old rat, fixed in Bouin. Only one kind of nucleus is apparent. The nuclei show some crenation. H. & E. ×1200.

Fig. 3. Transverse section of the nuclei of ameloblast layer from lower incisor of 2-day-old rat, fixed in Zenker. All the nuclei appear similar. They are densely stained, irregular in outline, and in places are bunched together. H. & E. ×1200.

Fig. 4. Transverse section of the nuclei of ameloblast layer from lower incisor of 2-day-old rat, fixed in Helly. All the nuclei appear similar, being large and rounded, though many of them have flattened outlines. H. & E. ×1200.

Fig. 5. Transverse section of the nuclei of ameloblast layer from lower incisor of 2-day-old rat, fixed in absolute alcohol. The nuclei are mainly large and rounded, but a number of smaller, more irregularly shaped nuclei, which are more deeply stained, can also be seen. H. & E. ×1200.

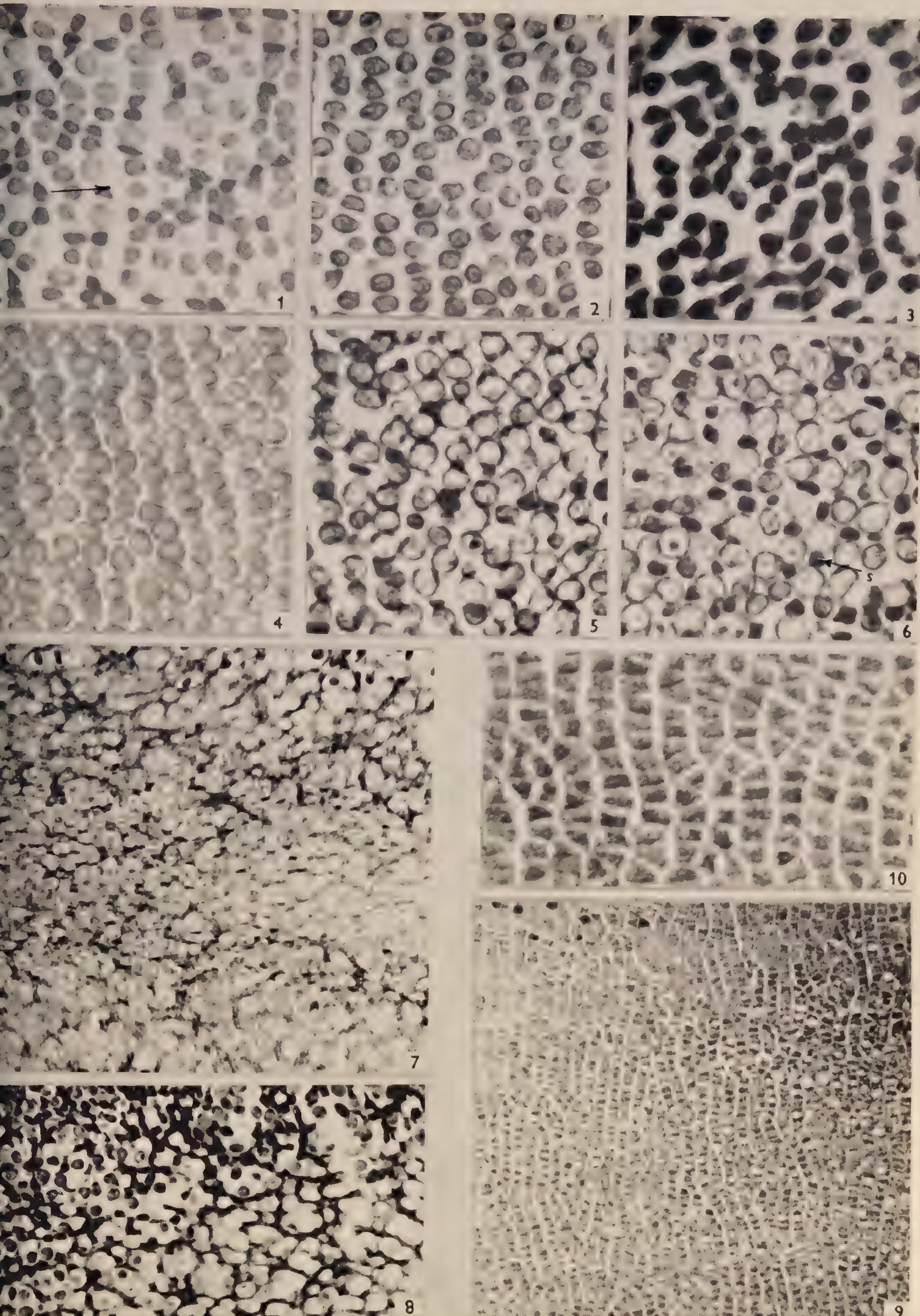
Fig. 6. Transverse section of the nuclei of internal enamel epithelium from lower incisor of 2-day-old rat, fixed in absolute alcohol. Two kinds of nuclei are present in nearly equal numbers; the first large and rounded, the second smaller, deeply stained and more irregular in shape. *s*, small dark nucleus. H. & E. ×1200.

Fig. 7. Tangential section of the ameloblast layer from lower incisor of 2-day-old rat, fixed in 40 % chilled formalin; in the centre of the field the cells of the ameloblast layer are cut transversely. Towards the right of centre the 'honeycomb' of the matrix of the developing enamel appears. At the top of the field a few nuclei of the cells of the ameloblast layer are visible. Two cytoplasmic elements can be seen, the darker-stained one seems to surround the lighter-stained one, and can be traced (at the distal end) in continuity with the matrix of the developing enamel. H. & E. ×460.

Fig. 8. Tangential section of the internal enamel epithelium from lower incisor of 2-day-old rat, fixed in 40% chilled formalin. In this field nuclear region and bodies of the cells are cut obliquely. Here the darker-stained nuclei are in almost equal numbers to the larger lighter-stained nuclei, and the darkly stained cytoplasmic element is very prominent. H. & E. ×460.

Fig. 9. Tangential section of the ameloblast layer from lower incisor of 2-day-old rat, fixed in Bouin. This section is at approximately the same position and level through the enamel organ as that shown in fig. 7. To the left of the centre of the field there are triangular and polygonal areas of cytoplasm, whereas at the centre and towards the right the cytoplasmic pattern is more irregular, being broken up by a large number of circular gaps. H. & E. ×460.

Fig. 10. A higher power view of the triangular and polygonal-shaped areas of cytoplasm which are seen in fig. 9. H. & E. ×1200.



SYMONS—Fixation on ameloblast layer of enamel organ in rat

(*Facing p.* 532)

OBSERVATIONS ON OSTEOGENESIS IN THE FEMUR OF THE FOETAL RAT

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INTRODUCTION

There is a voluminous literature on the histogenesis of bone, which has been reviewed by Retterer (1905), and Weidenreich (1930). In spite of this there does not appear to be any detailed account of the development of a mammalian long bone which covers the whole of the foetal period. The present account attempts to do this, and to relate certain of the phenomena of histogenesis to the growth processes of the bone.

Electron microscopy has made considerable advances in our knowledge of the fibrous structure of bone and has shown the relation of the fibres to mineralization of bone matrix. However, during the foetal period when mineralization is occurring for the first time, our knowledge of the fibrous structure of the matrix is not well known. In the subsequent account particular attention will be given to this latter aspect.

MATERIAL AND METHODS

Pregnancy was dated from the time of finding sperms in the vaginal smear, which was taken as the first day. Foetuses were obtained at daily intervals from the 17th day to the 23rd day (birth); in all, twelve animals were used. The right hind-limb was fixed in Bouin's fluid or Zenker-formaldehyde and embedded in paraffin. Serial longitudinal sections were cut at 5–7 μ and stained with Delafield's haematoxylin and eosin, Toluidin blue, Heidenhain's haematoxylin and van Gieson, or a modification of Long's reticulum silver impregnation, in which the final stages were omitted.

OBSERVATIONS

Seventeenth-day foetus

This stage is characterized by an intact cartilage model. The cells in the middle of the cartilaginous shaft are greatly hypertrophied (Text-fig. 1), but are not fragmented. Related to the middle of the shaft there is a well-defined fibrous perichondrium containing fibroblasts. These cells are large and elongated with long flattened nuclei, and lie with their long axis parallel to the long axis of the shaft (Pl. 1, fig. 1). Between the cells there is a fine plexus of longitudinally directed argyrophilic fibres (Pl. 1, fig. 2). The space between the fibrous perichondrium and the cartilage cells is packed with preosteoblasts (Pritchard, 1952), lying two or three cells deep. These latter cells are round, with a large central nucleus, and their cytoplasm is faintly basophilic (Pl. 1, fig. 1). A dense network of argyrophilic fibres exists between the deepest preosteoblasts and the cartilage cells. These fibres for the most part run

longitudinally, parallel to the cartilage surface, but a few fibres pass between adjacent preosteoblasts (Pl. 1, fig. 2).

Eighteenth-day foetus

The dense network of fibres seen round the shaft of the cartilage model in the previous stage has extended to cover the whole of the zone of hypertrophied cartilage cells. In haematoxylin and eosin preparations, the fibrous network is not apparent, and the bony matrix appears amorphous. This matrix has well-marked boundaries and will be referred to as the perichondrial collar of bone (Text-fig. 2). Some of the more central cells of the cartilage have commenced to disintegrate. Osteoblasts have appeared under the fibrous perichondrium and lie against the collar of fibres. It is usual to refer to the fibrous investing sheath as the periosteum once osteoblasts have appeared, and this term will be used now and in all later stages. Osteoblasts are distinguished by their irregular form, eccentric nucleus and a granular basophilic cytoplasm (Pl. 1, fig. 3). Occasional mitotic figures may be seen amongst the preosteoblasts. In the middle of the shaft, cells arising from the deepest layers of fibroblasts may be seen to be differentiating into preosteoblasts. This change is characterized by an enlargement of the nucleus, and the assumption of a spherical shape by both cytoplasm and nucleus. Occasional pleomorphic cells, with acidophilic cytoplasm and usually multinucleated, are to be seen amongst the osteoblasts and preosteoblasts (Pl. 1, fig. 4). They appear to be identical with the osteoclasts found in close relationship to bone in later stages. Capillaries or vascular spaces can be seen under the periosteum. The external surface of the collar of perichondrial bone is irregular, with osteoblasts lying in concavities. Spicules of bone matrix may be seen arising from this surface of the collar and passing between the osteoblasts (Pl. 1, fig. 3). The fibres of the matrix form a fine network, and run longitudinally. The deep surface of the collar is smooth and lies on a layer of flattened cartilage cells, with only a thin layer of cartilage matrix intervening. Interruption of the middle portion of the collar occurs at numerous points. Osteoclasts are to be seen at these points of interruption, wrapped round the free borders of the collar (Pl. 1, fig. 4). They may also be seen lying adjacent to the collar on its external surface in places where interruption has not yet occurred. Invasion of the cartilage is by means of a mass of mesenchymal cells, which are characterized by their irregular outline, indistinct cell boundary, faintly basophilic cytoplasm and a nucleus which is smaller than that of a preosteoblast (Pl. 1, fig. 5). The erosion of the cartilage matrix is confined to small foci immediately adjacent to the collar. It is interesting to note that the more peripheral cartilage cells are still intact and appear to be liberated when their capsule of matrix is perforated.

Nineteenth-day foetus

Invasion of the cartilage continues, but in the centre of the shaft a core of cells persists. These cells do not hypertrophy to the same extent as the other cartilage cells, and as they are liberated they escape into the invading mesenchyme (Pl. 1, fig. 6). Prominent amongst the cells of the primary marrow cavity are isolated mesenchymal cells, and cells similar to preosteoblasts. Mitotic figures may be seen in these cells. Capillaries have formed and are abundant, and are always to be found

close to the point of interruption of the capsule of a cartilage cell. Osteoclasts have appeared in the marrow and are usually found close to the isolated portions of cartilage matrix which have persisted. No recognizable osteoblasts are present in the marrow cavity and there is no evidence of bone matrix formation.

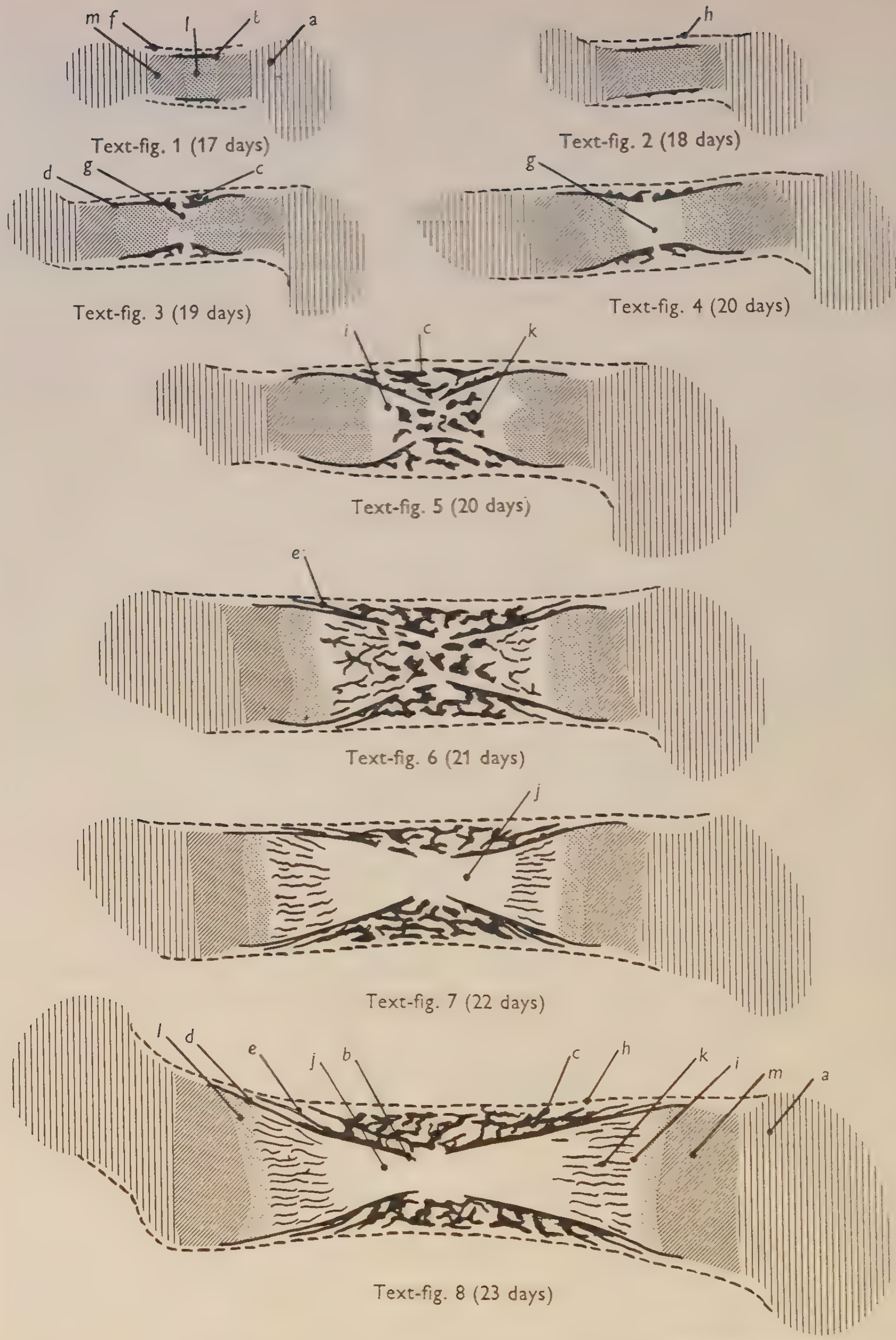
The collar extends at either end as far as the zone of flattened cartilage cells. The continued increase in the diameter of the shaft at the subepiphysial region results in a flaring of the extremities of the collar, and the collar acquires an hour-glass appearance. The concavity of the hour-glass is bridged by the fibrous periosteum (Text-fig. 3). The central portion of the collar, whose matrix contains the fine longitudinally running fibres is, for the most part, removed during the process of invasion. Silver impregnation shows that the more peripheral part of the collar, which is related to intact cartilage cells, has a different fibrous structure. Here the collar contains short, radially directed, fine bundles of fibres. They may appear in places to run obliquely towards the ends of the bone (Pl. 1, fig. 7), but usually they are at right angles to the surface of the diaphysis (Pl. 1, fig. 8), and give a brush-like appearance.

Trabeculae of bone matrix appear between the osteoblasts in the concavity of the hour-glass-shaped collar. They are confined to the area immediately adjacent to the remains of the collar, and appear continuous with the matrix of the collar. Silver impregnation shows this bone to have a characteristic structure consisting of a very fine network of fibre bundles (Pl. 1, figs. 9, 11). As the trabeculae extend, osteoblasts become enclosed in large oval lacunae. The intercellular formation and the fibrous structure of this bone distinguishes it from the perichondrial bone. It corresponds to the bone that is usually called periosteal bone, and this term will be used in the present account.

Twentieth-day foetus

The space between the fibrous periosteum and the perichondrial collar of bone becomes accentuated, and large vascular spaces appear between the osteoblasts (Text-fig. 4). In a more advanced specimen examined at this age, the trabeculae of periosteal bone have extended and have come to lie between the vascular spaces (Text-fig. 5). The formation of periosteal bone has progressed towards the extremities. The periosteal bone becomes deposited on the surface of the perichondrial bone, and osteoblasts come to lie in lacunae on the outer surface of the perichondrial bone.

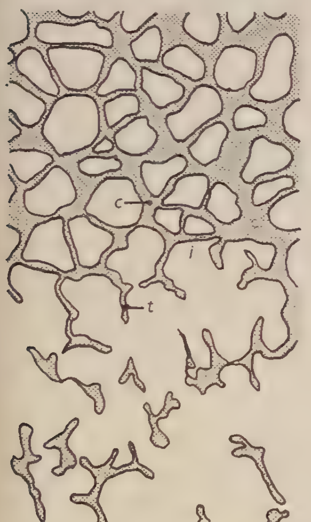
Invasion of the cartilage continues, and the central core of cells seen on the 19th day has disappeared. The hypertrophied cells are arranged in an irregular manner, and consequently the invasion does not form a regular front, but advances at isolated points. The invading tissues include numerous vascular spaces which may be seen entering the cartilage lacunae immediately following the interruption of the matrix. The cartilage cells have disintegrated before the invading tissues reach them. Large numbers of active osteoblasts are present in the marrow cavity and a thin layer of bone matrix is laid down on the persisting trabeculae of cartilage matrix. This endochondral bone shows a fibrous structure similar to periosteal bone, consisting of a fine felt-like plexus of argyrophilic fibres (Pl. 1, fig. 10). Deposition of bone never occurs in the newly formed primary spongiosa, and the invading buds are separated from the secondary spongiosa by a distance equivalent to the extent



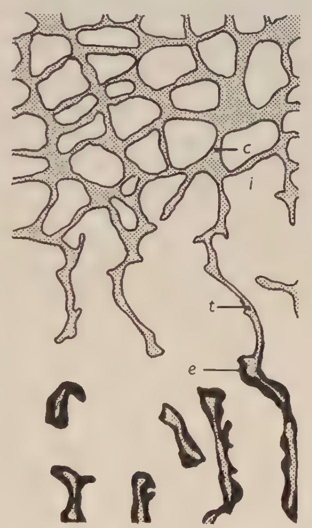
Text-figs. 1–8. Longitudinal sections through the femur of foetal rats to show the distribution of bone matrix. ×20 (semi-diagrammatic).

a. epiphysial cartilage
b. early perichondrial bone
c. early periosteal bone
d. late perichondrial bone
e. late periosteal bone
f. perichondrium
g. primary medullary cavity
h. periosteum
i. primary spongiosa
j. secondary medullary cavity
k. secondary spongiosa
l. zone of hypertrophied cartilage
m. zone of proliferating cartilage

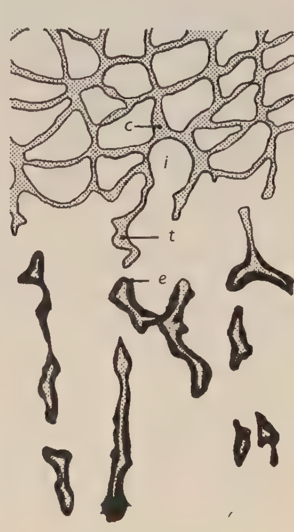
of at least six hypertrophied cartilage cells (Text-fig. 9). The zone of primary spongiosa contains large numbers of preosteoblasts. The irregular arrangement of the hypertrophied cartilage cells results in an unorganized appearance of both the primary and secondary spongiosa (Text-fig. 9), which is in contrast to the spongiosa of older individuals. The invasion of the cartilage lying immediately under the perichondrial collar of bone results in the persistence in places of a thin layer of cartilage matrix, which shows metachromasia with Toluidin blue, applied to the deep surface of the collar. This layer of cartilage matrix has a serrated deep surface due to the persistence of the bases of transverse trabeculae, which in places may be intact. In many places endochondral bone is laid down on this layer of cartilage matrix. In such situations the cartilage matrix appears as a cement line separating the perichondrial and endochondral bone, but its thickness and irregular deep surface distinguish it from a typical cement line (Pl. 1, fig. 11).



Text-fig. 9 (20 days)



Text-fig. 10 (21 days)



Text-fig. 11 (22 days)

Text-figs. 9–11. Longitudinal sections through the zone of endochondral ossification at the distal end of the rat's femur, which show the formation of the trabeculae of primary spongiosa (t), and their relation to the cartilage matrix (c). Note the relation of the deposition of endochondral bone (e) to the invasion front (i). ×210.

Twenty-first-day foetus

The perichondrial collar of bone continues to elongate and diverges widely at its extremities, the latter being evidence of the rapid increase in diameter of the shaft in the region of the hypertrophied cells. This in turn results in more extensive periosteal bone formation (Text-fig. 6).

The trabeculae of periosteal bone at the extremities of the diaphysis have a characteristic shape. They appear as linear structures, and are parallel to the surface of the underlying cartilage. Silver impregnation shows that the fibrous structure of these needle-like trabeculae differs from the periosteal bone of the centre of the shaft, as the former contain coarse fibre bundles of considerable length, which run in the

same direction as the trabeculae (Pl. 1, fig. 12). Lacunae are present which are elongated in the direction of the trabeculae and contain osteoblasts. There are large areas of matrix which appear homogeneous and apparently devoid of fibres. Such areas are extensive, and are as characteristic as the thick longitudinal fibre bundles.

The hypertrophied cartilage cells now form longitudinal rows or columns which arise in the zone of flattened cells, the latter had commenced to form short rows in the previous stage. The rows are closely packed, and the transverse and longitudinal trabeculae are of equal thickness. Row formation has two effects: first, the trabeculae of the primary spongiosa have a linear appearance (Text-fig. 10) as they are formed from the longitudinal trabeculae of the hypertrophied cartilage, and secondly, the invasion of the cartilage cells occurs along a regular front. Invasion occurs in 'bays' with the persistence of only some of the longitudinal trabeculae. The trabeculae of primary spongiosa are shorter than those seen at the last stage (Text-fig. 10), this suggests that invasion is outstripping the maturation of the cartilage. This suggestion is supported by the relatively shallow zone of hypertrophied cells as compared with the last stage.

Twenty-second-day foetus

Well-defined linear trabeculae of secondary spongiosa are present. The primary spongiosa is very reduced as the deposition of bone closely follows the invasion (Text-fig. 11). The irregular trabeculae of secondary spongiosa in the centre of the shaft are being eroded, with the formation of a secondary medullary cavity (Text-fig. 7). Large numbers of osteoclasts are present in the area of erosion, of which some are free in the marrow cavity, but the majority are closely applied to trabeculae of bone.

Twenty-third-day foetus

The collar has more than doubled its length since the twentieth day and its shape suggests that it has lengthened equally at either end (Text-fig. 8). The erosion associated with the formation of the secondary medullary cavity has resulted in the destruction of the central part of the collar and the immediately adjacent trabeculae of periosteal bone.

The most recently formed periosteal bone in the middle of the shaft still has the same fibrous structure as seen at 19 days, that is, a fine fibre network. The continued deposition of bone matrix has resulted in the incorporation of osteocytes within the trabeculae. These cells have a faintly basophilic cytoplasm and are smaller than osteoblasts. They occupy lacunae which are irregular in form and in distribution; canaliculi are few and are tortuous. The periosteal bone at the extremity of the diaphysis continues to form as linear trabeculae, containing coarse longitudinal fibre bundles.

The secondary medullary cavity has increased in extent since the last stage and has limited the secondary spongiosa to the subepiphysial zones.

DISCUSSION

(*a*) *Foetal pattern of growth*

Bhaskar, Weinmann and Schour (1954) showed an initial rapid growth in length of the tibia of a mutant strain of rats, which slowed down on the 20th day. Moss, Noback & Robertson (1955) observed a decrease in the specific growth rate of human long bones at the 12th week, and associated this with the findings of Blechschmidt (1951) who showed that the longitudinal arrangement of hypertrophied cartilage cells appeared at this stage. Thus the appearance of columns of cartilage cells in the femur of the rat, which occurs betweeen the 20th and 21st days, probably marks the end of a period of rapid elongation.

The relative shortening of the trabeculae of the primary spongiosa after the 20th day also suggests that the rate of growth is decreasing. The form of the perichondrial collar would indicate that both epiphysial plates contribute equally to its growth in length. It is difficult to investigate this experimentally but Bhaskar, Weinmann & Schour (1954) identified the centre of the shaft by means of the constriction of the collar, in a strain of mutant rats in which the collar persists, and showed elongation occurred equally at either end in both the foetal and the neonatal tibia. Bisgard & Bisgard (1935) administered phosphorus to pregnant goats. They showed, radiologically, lines of increased density at equal distances from the centre of the foetal long bones. The centre was determined by the course of the nutrient vessel.

(*b*) *Histogenesis of bone in the foetal diaphysis*

In this account it has not been possible to distinguish between preosseus tissue and bone, as the material was decalcified. In the past such a distinction has often been made, but Bloom & Bloom (1940) showed that uncalcified bone matrix (osteoid) was rarely seen in normal osteogenesis. Electron microscopy of endochondral osteogenesis in the neonatal kitten by Scott & Pease (1956) showed a narrow zone of uncalcified bone matrix immediately adjacent to osteoblasts. The present investigation shows no apparent difference between the fibrous structure of newly formed bone matrix and that of older areas. This would suggest that the fibrous matrix is always laid down in its final form, and the concept of a preosseus fibrous matrix is not a morphological fact. It is of particular significance that Scott & Pease (1956) showed the 'preosseous fibrous zone' to consist entirely of bundles of mature collagen fibres.

The perichondrial collar, the first lamella of bone to appear, is of great interest. Lacroix (1945), basing his evidence on transplantation experiments, concluded that its formation is induced by the hypertrophic cartilage. Little attention has been given to its histogenesis, and its first appearance requires comment. The fibrous matrix is formed before the appearance of fully differentiated osteoblasts. Pritchard (1952) recognized this phase of osteogenesis when he described, in the mandible of the foetal rat, a zone of preosteoblasts lying in a network of fine fibres, but at this stage osteoblasts were also present. There is no evidence in the rat to support Carey's (1922) account of the formation of the collar in the pig's femur, where he observed the outer cells of the cartilaginous shaft apparently differentiated into osteoblasts and helped to form the perichondrial collar.

The fibrous structure of perichondrial bone in the human foetal femur was described by Weidenreich (1930) as consisting of coarse fibre bundles arranged parallel to the longitudinal axis of the bone. Such a description might apply to the earliest matrix of the collar of the rat, except that the fibres form a fine plexus rather than coarse bundles. It is perhaps surprising that the fibrous structure of the later perichondrial bone, with its characteristic brush-like appearance, has apparently escaped recognition in the past. There is a well-defined hyaline border on the deep surface of the collar, which usually survives invasion of the cartilage. Weidenreich (1930) observed the collar separating the endochondral from the periosteal bone, and described this amorphous internal border of the collar, but did not comment on its origin.

The earliest periosteal bone has a distinct fibre structure consisting of a woven plexus of fine fibres. It continues to form throughout the foetal period and results in a loose trabecular structure. It corresponds to the early embryonic woven bone described in the femur of the human embryo by Zawisch-Ossenitz (1929).

The longitudinal trabeculae that appear at the extremities of the diaphysis form a distinct type of periosteal bone. They have a characteristic structure of long coarse fibre bundles and areas apparently devoid of fibres. This bone was recognized by Zawisch-Ossenitz (1929) and termed late embryonic woven bone but no detailed account was given of its structure. Weidenreich (1930) discussed the formation of this type of bone and denied the presence of osteoblasts, claiming that connective tissue cells become transformed into osteocytes.

The relationship between the calcification of the cartilage matrix and its invasion was described by Bloom & Bloom (1940). They showed that in rat long bones perichondrial ossification occurred before calcification of the matrix, the latter usually commencing in the centre of the shaft. Streeter (1949) described the erosion of the cartilage in the human humerus, but did not describe perforation of the collar by osteoclasts, which would seem to be the case in the rat. The appearance of osteoclasts in the subperichondrial space at this stage raises the problem of the origin of these cells. It would seem that they arise locally but, as Hancox (1949, 1956) stated, the origin of these cells is still an open question. The initial invasion of the cartilage is by means of a syncytium of undifferentiated mesenchymal cells, the 'osteogenic mesenchyme' of Stump (1925). Capillaries rapidly develop *in situ* and are responsible for invasion. The part played by osteoclasts in the erosion of the cartilage matrix was described by Dodds (1932), who associated them not with the initial invasion but with the removal of remains of calcified cartilage.

The onset of endochondral ossification does not immediately follow invasion. In the rat 2 days elapse before osteoblasts appear in the primary marrow cavity. In man invasion of the humerus occurs at the end of the 7th week, but no endochondral bone formation took place until the 10th week (Gardner, 1956). The earliest trabeculae are the remains of matrix formed during the initial rapid period of growth and are consequently irregular, but after the 20th day they are linear following the columnar arrangement of the cartilage cells. The proximity of the deposition of bone matrix to the invading cells would appear to be an index of the rate of maturation of the cartilage cells. The bone formed within the marrow cavity has a matrix consisting of a fine woven network of argyrophilic fibres.

(*c*) *Formation of a secondary medullary cavity*

The secondary medullary cavity results from the endosteal erosion of the bone, and is associated with the appearance of large numbers of osteoclasts in the area. It commences in the centre of the shaft with the destruction of the secondary spongiosa and progresses towards the extremities, with the result that the secondary spongiosa is confined to the subepiphysial region. The initial destruction involves the waist of the perichondrial collar and the deeper trabeculae of periosteal bone. Bhaskar, Weinmann, Schour & Greep (1950) showed it to occur in the tibia of the rat on the 22nd day, which is the same time as it occurs in the femur. The pattern of osteogenesis that results after the occurrence of this phenomenon, namely periosteal deposition of bone, subepiphysial endochondral bone formation, and endosteal erosion, was shown in the autoradiographic studies of new-born rats by Leblond, Wilkinson, Belanger, and Robichon (1950) and Greulich & Leblond (1953).

It is surprising that this important incident in the development of a bone has received so little attention. It is known that it must occur, but rarely can its occurrence be timed. The factors responsible for the initiation of the process are unknown, but if it is a generalized phenomenon occurring in all bones that have reached a certain stage of development, then a hormonal mechanism is a possibility. Sometimes endosteal erosion fails to occur, and there is an extensive literature on such amedullary bones. A lack of understanding of normal osteogenesis has in many cases led writers to fail to distinguish between the formation of abnormal endochondral trabeculae which apparently resist resorption, and the non-appearance of the normal developmental erosive process.

Gruneberg (1935, 1936, 1938, 1943) has shown a failure of medullary erosion in a strain of mutant mice. In these 'grey lethal' mice large numbers of medullary osteoclasts were present. A similar condition was also found by Gruneberg (1948) in a strain of microphthalmic mice. In both these mutant strains death invariably occurred shortly after the third week of postnatal life. A similar though temporary condition was found in the long bones of certain mutant rats. Bhaskar, Weinmann, Schour & Greep (1950) showed in these 'incisor-less animals' the persistence of the secondary spongiosa and the perichondrial collar of the tibia. Endosteal erosion in these animals was only delayed, for it appeared between 30 and 50 days of postnatal life. It is unfortunate that the histogenesis of the endochondral bone in these conditions has not been described.

Osteopetrosis (Albers-Schönberg's Disease) in man has amongst its principal features the failure of endosteal resorption. Cohen (1951) described a typical example where the shaft was filled with spongiosa and the perichondrial collar was intact though the child was 32 months old. Zawisch (1947) observed the irregular invasion of the hypertrophied cartilage cells and the atypical structure of the spongiosa. Pearce (1948, 1950) described hereditary osteopetrosis in the rabbit when death invariably occurred about the 4th week of postnatal life. The condition features persistence of the spongiosa of the long bones and atypical endochondral bone formation. In this connexion it is interesting to note that there is failure of medullary erosion normally, in certain, if not all of the long bones of the Sirenia and the condition persists throughout adult life. Fawcett (1942) described the structure

of the rib and humerus of the adult and foetal manatee and, as in osteopetrosis, abnormal endochondral bone formation was observed, but details were not given. It would be interesting to know if amedullary bones are found in other aquatic mammals.

SUMMARY

1. Osteogenesis, with special reference to the fibrous structure of the bone matrix, is described in the femur of the foetal rat.

2. The earliest perichondrial bone appears on the 17th day (i.e. 16 days after finding sperms in a vaginal smear). This bone has a finely woven fibrous structure and at first its formation is associated with preosteoblasts alone.

3. The later perichondrial bone which is found from the 19th day onwards is associated with osteoblasts and has a characteristic fibrous structure consisting of short, fine, radially directed fibre bundles, which give a brush-like appearance.

4. The earliest periosteal bone which appears on the 19th day forms both on the surface of the perichondrial bone, and as irregular subperiosteal trabeculae. It has a fine woven fibrous structure.

5. Elongated trabeculae of periosteal bone appear at the extremities of the diaphysis on the 21st day. They contain longitudinally running, coarse fibre bundles and amorphous areas.

6. Endochondral bone appears on the 20th day and has a finely woven fibrous structure.

7. Interruption of the perichondrial bone and invasion of the cartilage occurs on the 18th day.

8. The formation of irregular primary spongiosa ceases, and linear trabeculae appear on the 21st day.

9. The secondary spongiosa commences to disappear in the centre of the shaft on the 22nd day.

10. These findings are discussed in relation to the growth changes occurring in the bone during development.

ACKNOWLEDGEMENTS

The author is indebted to Prof. J. D. Boyd for his helpful criticism. He is also grateful for the technical assistance of Mr J. W. Cash and Miss C. J. Hitchins, and to Mr J. F. Crane for the photography.

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EXPLANATION OF PLATE

Longitudinal sections of the femur of the rat, all are orientated in the same manner.

Fig. 1. Seventeenth-day foetus (Haematoxylin and Eosin). ×780. The perichondrium contains elongated fibroblasts (*a*); between these and the cartilage (*b*) are numerous preosteoblasts (*c*).

Fig. 2. Seventeenth-day foetus (Long's method). ×780. A plexus of fine argyrophylic fibres (*a*) lies between the preosteoblasts (*b*) and the cartilage. The longitudinally directed fibres of the perichondrium (*c*) are visible.

Fig. 3. Eighteenth-day foetus (H. and E.). ×780. Osteoblasts (*a*) lie under the perichondrium (*b*). Bone matrix (*c*) is visible between the osteoblasts and the cartilage.

Fig. 4. Eighteenth-day foetus (H. and E.). ×780. An osteoclast (*a*) is seen at the point of interruption of the collar. A preosteoblast (*b*) is undergoing mitosis.

Fig. 5. Eighteenth-day foetus (H. and E.). ×780. A bud of mesenchymal cells (*a*) is invading the cartilage. Note the syncytial appearance.

Fig. 6. Nineteenth-day foetus (H. and E.). ×780. The formation of a primary medullary cavity. The invading tissue contains capillaries (*a*) and mesenchymal cells (*b*).

Fig. 7. Twentieth-day foetus (Long's method). ×780. The obliquely directed fibres of the perichondrial collar (*a*) can be seen between the zone of flattened cartilage cells and the preosteoblasts. Note the coarsely bundled fibres of the perichondrium (*b*).

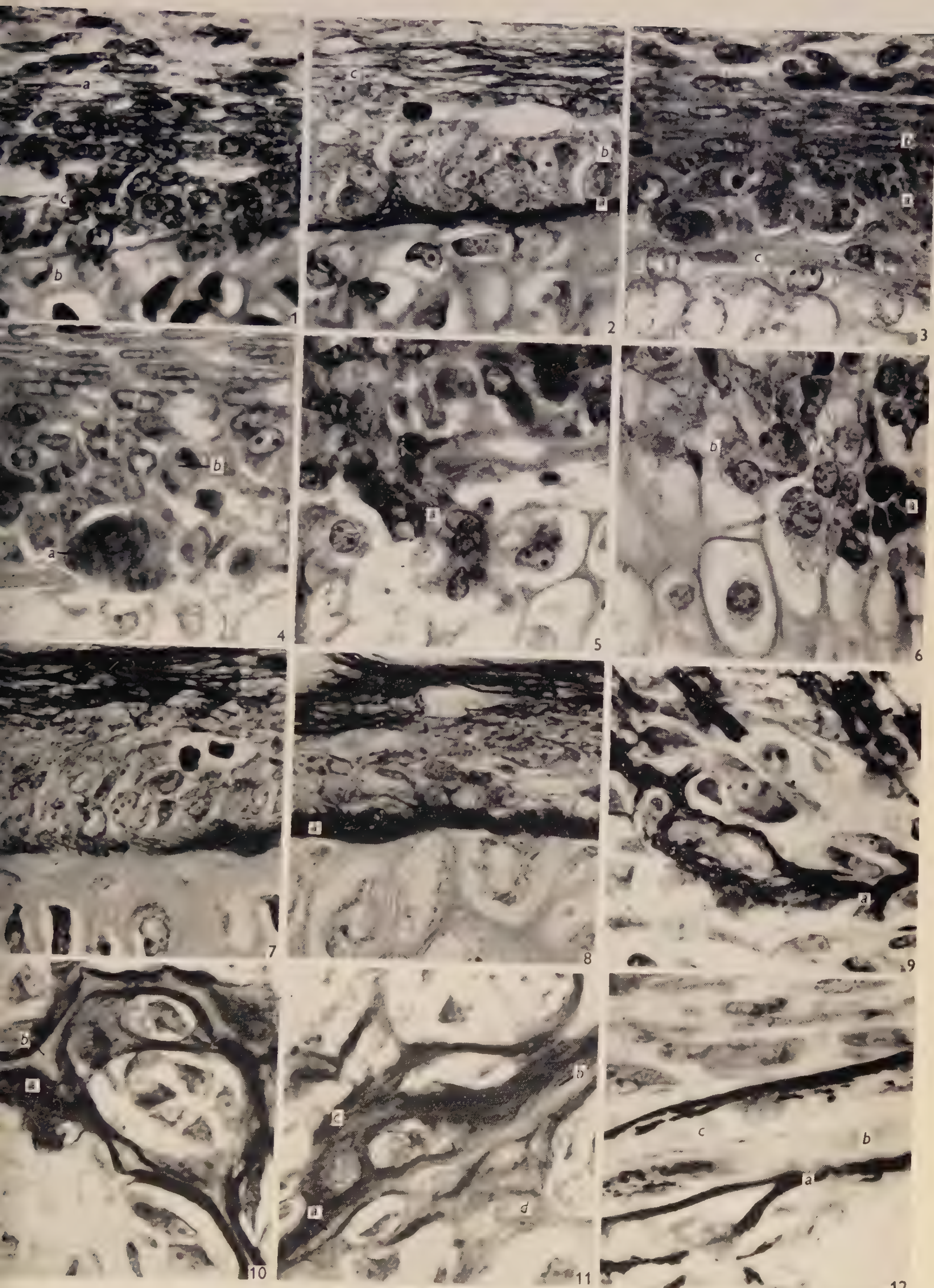
Fig. 8. Twentieth-day foetus (Long's method). ×780. The palisade of short fine bundles of fibres of the perichondrial collar (*a*) can be seen between the osteoblasts and the hypertrophied cartilage cells.

Fig. 9. Twentieth-day foetus (Long's method). ×780. The early periosteal bone (*a*) has a loose trabecular structure. The finely woven fibres of the bone matrix collect in some places into thicker bundles.

Fig. 10. Twentieth-day foetus (Long's method). ×780. The fine fibred endochondral bone matrix (*a*) can be seen lining the remains of the cartilage matrix (*b*).

Fig. 11. Twentieth-day foetus (Long's method). ×780. The diagonal, apparently fibre-free band (*a*) represents the cartilage matrix that lines the perichondrial collar (*b*), these separate the finely fibred matrix of the periosteal bone (*c*) and the endochondral bone (*d*).

Fig. 12. Twenty-second day foetus (Long's method). ×780. The fibres of the late periosteal bone matrix form thick longitudinally running bundles (*a*), between these may be seen apparently fibreless matrix (*b*) which contains lacunae (*c*).



PRATT—OSTEOGENESIS IN THE FEMUR OF THE FOETAL RAT

(*Facing p.* 544)

THE FORCES OPERATING AT THE HUMAN ANKLE JOINT DURING STANDING

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INTRODUCTION

In recent years it has become generally appreciated (Clemmesen, 1951 and Fulton, 1949) that the ankle joint is stabilized during standing by two forces operating in harmony. One is that exerted by the postural activity in the posterior crural muscles, and the other is the passive resistance to dorsi-flexion which is exerted by the tissues of the same region. Passive resistance to joint movement by elastic tension in such tissues as joint ligaments, deep fascia, skin and the fibrous framework of muscles itself occurs over a certain terminal range of movement at every joint; its properties have been considered in some detail in a previous publication (Smith, 1956).

The postural activity in the posterior crural muscles during standing has been studied frequently by electromyography, and an analysis of the results of these investigations will be considered in this paper, but it is believed that this method cannot give an absolute quantitative value to the tension exerted by the muscles. On the other hand, the passive resistance of the tissues to dorsi-flexion at the ankle joint has not been previously studied. In the present investigation the contributions made by these two factors to the stability of the ankle joint during standing have been determined.

It is well known that during standing the centre of gravity oscillates in the sagittal plane. It moves in an arc around the axes of rotation of the ankle joints with an angular velocity which varies continually both in magnitude and direction. It is evident that such movement must be both motivated and controlled by the 'turning moments' or 'torques' which act on the body about the ankle axes and these torques are indicated in Text-fig. 1. In this diagram two lines have been constructed. One extends downwards and slightly backwards between the centre of gravity of the body and the ankle axis, and is L ft. in length. The other extends vertically downwards from the centre of gravity; it makes an angle of P° with the first line and passes F in. in front of the ankle axis. The force of gravity tends to cause downward displacement of the centre of gravity of the body at an acceleration which is customarily indicated by g and has a value of 32·2 ft. per sec. per sec. A force, by definition, is equal to the product of the mass of the object on which it acts and the linear acceleration it produces, and therefore if the mass of the human body—which is visualized as being concentrated at the centre of gravity—is M lb., the force exerted on the body by gravity is Mg poundals.

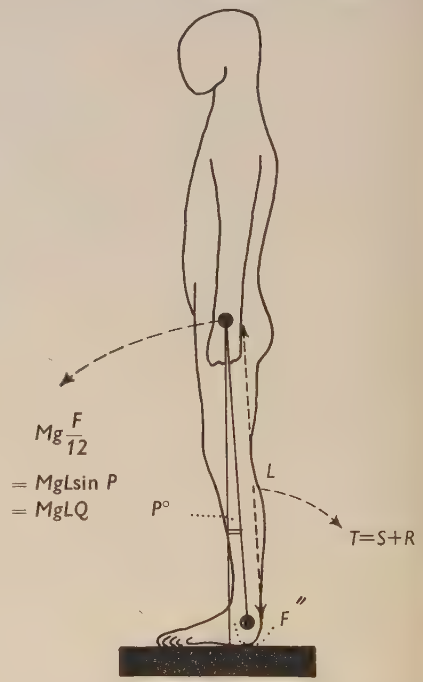
The torque or turning moment which a force exerts about a given axis is equal to the product of the force in poundals and the distance of the force from the axis in feet. Thus in Text-fig. 1 the dorsi-flexing torque exerted at the ankle axis by the

force of gravity is $Mg\,(F/12)$ poundals ft. From the same figure it will be evident that because $F/12 = L \sin P$, the torque can be alternatively expressed as $MgL \sin P$. Furthermore, in the consideration of angular motion it is often necessary to evaluate the angle P in radians rather than degrees. Transference between the two units is facilitated because during normal standing the angle P which represents body sway never exceeds about 6° (Hellebrandt & Braun, 1939); and consultation of standard mathematical tables shows that for angles of this size the radian measure is numerically equal to the sine of the angle. There are therefore three ways of expressing the dorsi-flexing torque exerted by gravity about the ankle axis, namely

$$Mg(F/12) = MgL \sin P = MgLQ \text{ poundals ft.}$$

when Q is the angle P in radians.

Opposing this dorsi-flexing torque is the plantar-flexing torque exerted by the tissues of the posterior crural regions of both lower limbs. This may be designated T poundals ft., and, as has been stated already, it is the sum of the plantar-flexing torques exerted by postural contraction in the posterior crural muscles (S poundals ft.) and by the passive tension in the tissues of the same region (R poundals ft.) i.e. $T = S + R$. The plantar-flexing and dorsi-flexing torques about the ankle joint operate in different directions and this fact must be indicated by a difference in sign. It is immaterial what convention is employed, but in this paper a dorsi-flexing torque is regarded as negative and a plantar-flexing torque as positive. Thus the resultant torque acting about the ankle joint is $(T - MgLQ)$ poundals ft.



Text-fig. 1. The torques acting about the ankle joint in standing. The upper marker represents the centre of gravity of the body and the lower marker the axis of rotation of the ankle joint.

This resultant torque tends to cause an angular acceleration of the centre of gravity about the ankle axis which may be designated (B) radians per sec. per sec. The actual value of (B) depends on the value of the factor known as the moment of inertia of the body about the ankle axis (I_a). The name of this factor has frequently given rise to misunderstandings: in the present context the moment of inertia is best regarded as being the equivalent, in relation to angular motion, of the mass in relation to linear motion. Thus it has already been noted that in linear motion, force = mass × linear acceleration: in the same way in angular motion, torque = moment of inertia × angular acceleration. That is

$$T - MgLQ = I_a B$$

$$S + R = MgLQ + I_a B \text{ poundals ft.}$$

In this equation the factors (S) and (R) are expressed in poundals ft. By dividing the terms on the right hand side of this equation by g, (S) and (R) can be expressed

in the more familiar non-gravitational units of pounds ft., 1 lb.ft. being the torque produced by a mass of 1 lb. acting at a distance of 1 ft. from an axis of rotation,

i.e. $$S+R=MLQ+\frac{I_aB}{g}\text{ lb.ft.,}$$

or $$S=MLQ+\frac{I_aB}{g}-R\text{ lb.ft.} \tag{1}$$

In this equation, M, L, I_a and g are constants for any one subject whereas S, Q, R and B vary with time. The solving of the equation necessitates the measurement of M and L and the experimental determination of I_a, R, Q, B and S.

METHODS

(1) *Determination of the position of the centre of gravity of the body*

In the present investigation it was not necessary to know the exact position of the centre of gravity in three dimensions; what was required was the distance between the centre of gravity and the ankle axis (L ft.).

This distance was determined by placing each subject supine on a board tilting freely about a transverse axis, so that the feet were at right angles to the legs and the arms were by the side. The position of the body in relation to the tilting axis was then varied until the position of balance was obtained.

(2) *Experimental determination of the moment of inertia of the body about the axis of the ankle joint* (I_a)

One of the standard methods for determining the moments of inertia of objects of irregular outline and composition is to regard the object as a compound or irregular pendulum. The duration in seconds of one complete oscillation of such a pendulum is

$$t=2\pi\sqrt{\frac{I}{MgD}} \tag{2}$$

I being the moment of inertia about the point of suspension and D the distance in feet between the point of suspension and the centre of gravity of the body. Thus if an object is suspended and allowed to swing through a small angle, the value of t can be determined and the moment of inertia about the axis of suspension calculated.

Direct determination of the moment of inertia of the living body about the ankle axis by this method would require suspension of the subject upside down about this axis. Use of the theorem of parallel axes makes this procedure unnecessary. This theorem states that if the moment of inertia about a given axis is I and the distance of that axis from the centre of gravity is D ft., then the moment of inertia about the centre of gravity itself is $I_c=I-MD^2$.

Furthermore, by the same theorem the moment of inertia about the ankle axis is

$$I_a=I_c+ML^2$$

and therefore $$I_a=I+M(L^2-D^2). \tag{3}$$

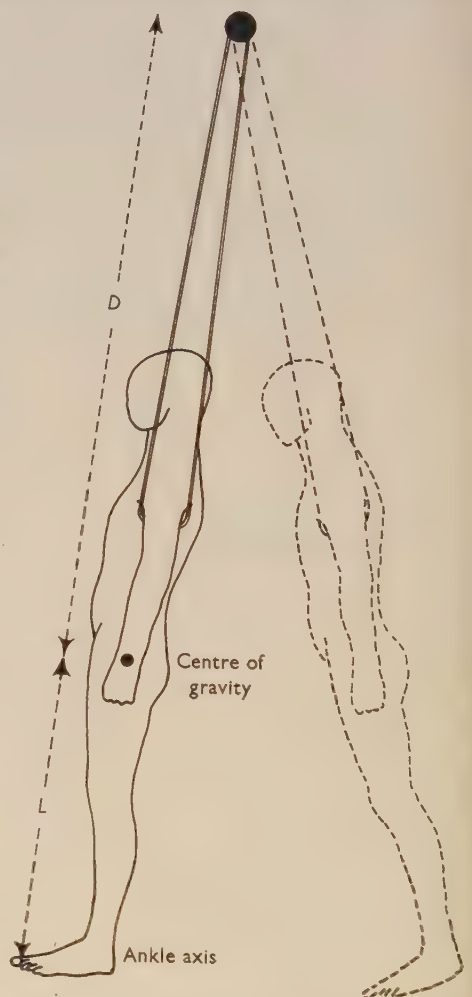
Thus each subject was suspended as in Text-fig. 2 from a beam by ropes passing beneath the axillae, and allowed to swing through a small arc. The average value of 't' for fifty oscillations was observed. The values of I about the axis of the beam was then determined from equation (2) and subsequently the value of I_a was calculated from equation (3).

(3) *The determination of the plantar-flexing torque which is exerted at the ankle joints during standing by the passive tension in the tissues in the posterior crural regions of both lower limbs* (R).

It is apparent that the value of this torque varies with the position of the ankle joint, increasing in dorsi-flexion and decreasing in plantar-flexion. Its values throughout the greater part of the range of movement at the ankle have been determined by the following method.

Each subject lay supine with the leg to be examined suspended clear of the table in a broad sling passing beneath the calf muscles (Text-fig. 3). The position of full voluntary dorsi-flexion at the ankle joint was then determined. Subsequently, a large sphygmomanometer cuff was applied to the mid-thigh and a pressure of 200 mm. Hg maintained in it until sensory and motor paralysis below the calf were complete. It was assumed that in these circumstances the posterior crural muscles reacted to stretch in the same way as denervated structures, an assumption which is in conformity with observations of Magun (1940) and Magladery, McDougal & Stoll (1950).

The foot was now moved slowly from plantar-flexion into dorsi-flexion by pulling on a calibrated tensile spring (C) attached to the foot in the region of the metatarsal heads (D). The movement was stopped some distance short of full voluntary dorsi-flexion to avoid injury to the tissues which had been rendered insensitive. During the movement a cine film was taken from the lateral side, the whole foot and leg and the calibrated spring being included in the field, and subsequently enlarged tracings were made of every fourth frame. On each tracing the lines AM and DM (Text-fig. 3) were constructed so that they passed from the anterior aspect of the patella



Text-fig. 2. Determination of the moment of inertia of the body about the ankle axis (I_a)

and from the attachment of the spring to the foot respectively, to the tip of the lateral malleolus which was regarded as lying on the axis of rotation of the ankle joint.

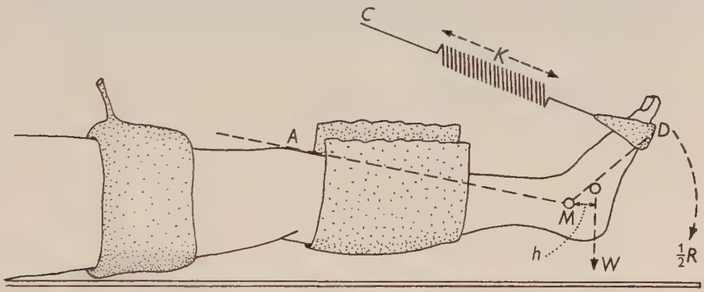
In these circumstances three torques acted about the axis of the ankle joint. If the tension in the spring is denoted by K, the dorsi-flexing torque exerted by the spring was the product of that tension and the minimum distance of the spring from the ankle axis, that is $K.DM.\sin \angle CDM$. The plantar-flexing torque due to the passive tension in the posterior crural tissues of the one limb was $\frac{1}{2}R$. The similar torque due to the weight of the foot was (Wh), W being the weight of the foot and h the horizontal distance between the centre of gravity of the foot and the ankle axis. At any moment during the experiment these torques were in equilibrium and therefore

$$K.DM \sin \angle CDM = \tfrac{1}{2}R + Wh,$$

or

$$\tfrac{1}{2}R = K.DM \sin \angle CDM - Wh. \tag{4}$$

Now the value of the factor Wh in this equation varies with the angulation of the ankle joint. It is evident from Text-fig. 3 that in plantar-flexion the factor Wh is



Text-fig. 3. Method for determination of values of R.

comparatively large because the distance h is large, but that, as the foot is dorsi-flexed the factor becomes progressively smaller until, when the foot is at right angles to the leg it approaches zero. In the present study the significant values of R are those operative during standing, when the angle between the leg and foot is rather less than 90°, and when the value of the factor Wh is consequently approximately zero. In this investigation, therefore, the factor Wh can be ignored and equation (4) can be approximated to

$$R = 2K.DM.\sin \angle CDM. \tag{5}$$

The values of K, DM, $\angle CDM$ and $\angle AMD$ were therefore measured on each of the tracings described above and a graph was then constructed showing the values of R for successive values of $\angle AMD$.

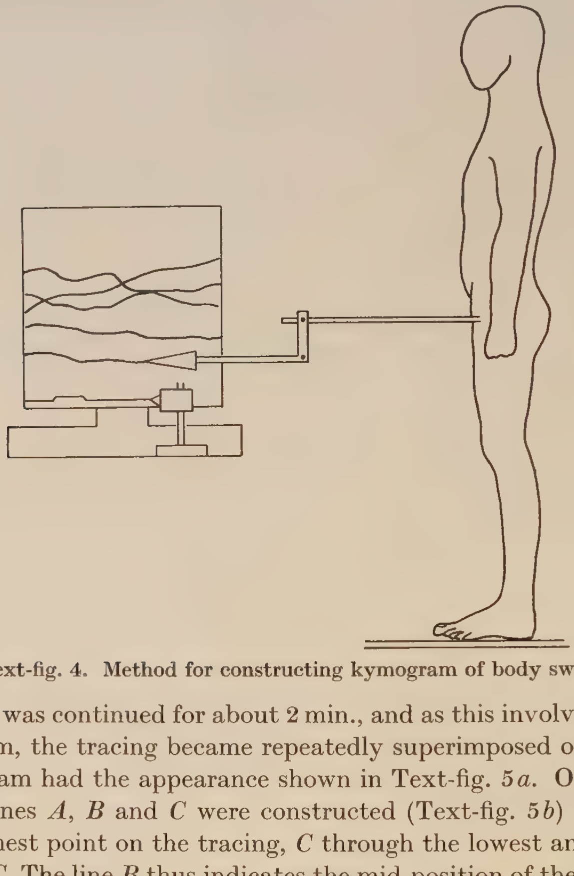
In standing the angle AMD is variable—it is in fact an inverse function of the angle P in Text-fig. 1. However, the variation is always small, being of the order of about 3°, and it has been found in practice that the associated variation in the value of R is insignificant. It is, therefore, justifiable to determine a single value of $\angle AMD$ from a lateral photograph of the standing subject, and to read off the

$R/\angle AMD$ graph the corresponding single value of R. This value of R, with only a slight degree of error, is applicable to all phases of standing in that subject.

(4) *Determination of variations in the angle P and in the angular acceleration B during stance*

The manner in which the terms MLQ and I_aB/g in equation (2) vary with time can be determined by the one set of experiments.

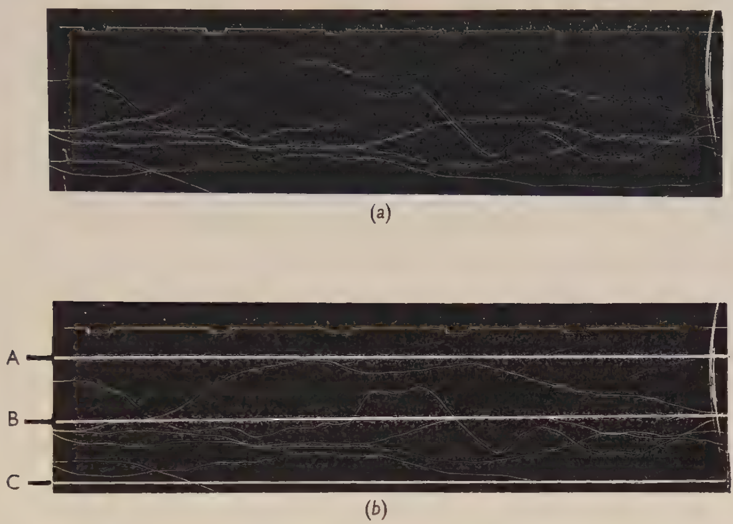
In each subject a magnified kymographic record of the antero-posterior movements of the centre of gravity during standing was prepared with an accompanying appropriate time tracing. The arrangement is shown diagrammatically in Text-fig. 4.



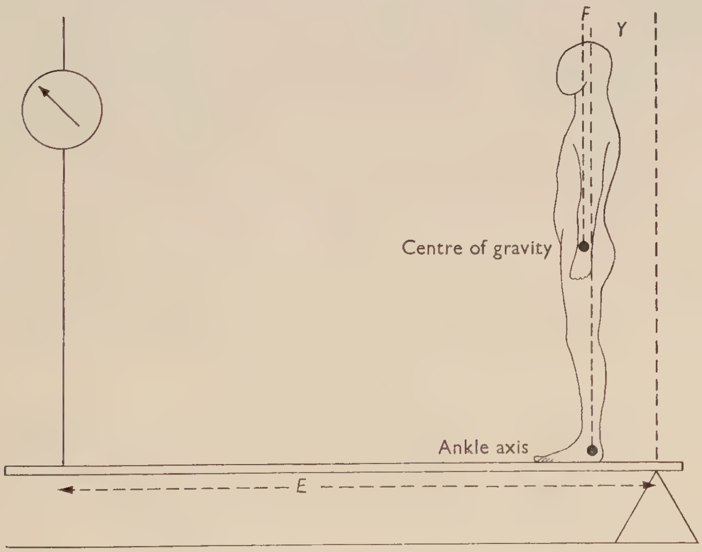
Text-fig. 4. Method for constructing kymogram of body sway.

The recording was continued for about 2 min., and as this involved several revolutions of the drum, the tracing became repeatedly superimposed on itself. Thus the finished kymogram had the appearance shown in Text-fig. 5*a*. On this kymogram the horizontal lines A, B and C were constructed (Text-fig. 5*b*) so that A passed through the highest point on the tracing, C through the lowest and B lay mid-way between A and C. The line B thus indicates the mid-position of the centre of gravity during the period of the recording: displacement of the tracing above the line B indicates a backward displacement of the centre from its mid-position, whereas displacement below the line indicates a forward displacement of the centre from the mid-position. Subsequently, the kymogram was photographed and the negative was projected at a high magnification on to 1 by $\frac{1}{10}$ in. graph paper on which random segments of the tracing plus the line B were drawn. In a typical example the final magnification of the displacements of the centre of gravity was $\times 92{\cdot}8$ and the time tracing was 1 sec. to 25·4 in.

In this form the tracing shows accurately the antero-posterior displacements of the centre of gravity in relation to the mid-position (line B), but it gives no absolute information because the relationship between this mid-position and the ankle axis



Text-fig. 5. (*a*) **Kymogram of body sway. 1 sec. time tracing.** (*b*) **The same kymogram with the lines** A, B **and** C **constructed.**



Text-fig. 6. Method of determining the relationship of the line B **in Text-fig. 5*b* to the axis of the ankle joint.**

is unknown. This relationship was established by use of the apparatus shown diagrammatically in Text-fig. 6.

A broad wooden plank about 6 ft. long was supported close to one end by a fulcrum and suspended at the other from a spring balance. The distance from fulcrum

to suspension may be designated E inches and the reading on the spring balance H_1 lb. The subject of body weight W lb. stood on the plank close to the fulcrum and facing the suspension so that the tip of the lateral malleolus (representing the ankle axis) was Y in. from the fulcrum.

Thereafter, if at any given moment the reading on the spring balance was H_2 lb., then the horizontal distance of the centre of gravity in front of the ankle at that moment was

$$F = \frac{E\,(H_2 - H_1)}{W} - Y.$$

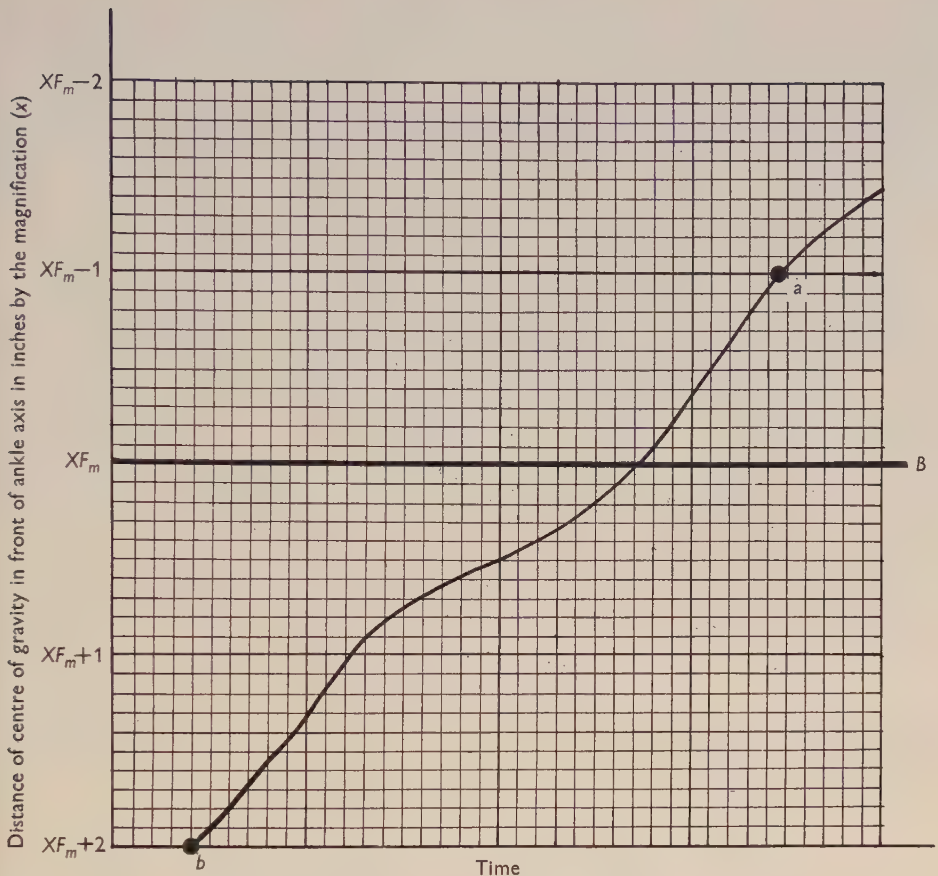
Each subject stood comfortably on this apparatus for about 2 min. and during this period the reading on the spring balance was continually recorded on cine film. From this film the maximum and minimum readings were noted (i.e. the maximum and minimum values of H_2) and from these the mid-reading was calculated and the mid-position of the centre of gravity in front of the ankle axis (F_m in.) was determined.

It has been noted by Hellebrandt & Braun (1939) that the mid-position of the centre of gravity remains practically constant during a stance of any appreciable duration, and it can therefore be said that the line B on the enlarged tracing of the kymogram represents a position of the centre of gravity F_m in. in front of the ankle axis. However, the magnification of this tracing must be borne in mind and it will be evident that if this magnification is denoted by X, then the position of the line B would, in this tracing, be XF_m in. from the ankle axis (Text-fig. 7). Furthermore, it has been noted that a displacement of the tracing above the line B represents a proportionate backward movement of the centre of gravity and vice versa. Thus, a point on the tracing such as (a) in Text-fig. 7 is 1 in. above the line B; consequently, at the magnification of the tracing it denotes a position of the centre of gravity $(XF_m - 1)$ in. in front of the ankle axis. Similarly, a point such as (b) in Text-fig. 7 is 2 in. below the line B; it therefore represents, at the magnification of the tracing, a position of the centre of gravity which is $(XF_m + 2)$ in. in front of the ankle axis. In this way the whole tracing was calibrated.

Thereafter the vertical positions of successive points on the tracing—which represent the distances of successive positions of the centre of gravity in front of the ankle axis (F) at a magnification X—were read off at intervals of 2·5 small squares, that is at intervals of the order of $\frac{1}{100}$ sec. The distances were tabulated as in column 2, Table 1.

It was previously stated (p. 546) that the term MLQ is equal to $ML \sin P$ which in turn is equal to $MF/12$ lb.ft. Thus, if each figure in column 2 is multiplied by $M/12X$ the values of MLQ are obtained at the same intervals of $\frac{1}{100}$ sec. and these are tabulated as in column 3.

Angular velocity is the angle traversed by a rotating body in radians divided by the time in seconds and is expressed in radians per second. In the present experiment it will be evident from Text-fig. 8 that at the end of the first $\frac{1}{100}$ sec. of the tracing the sine of the angle P_1 is $F_1/12L$, and that P_1 has therefore a value of $F_1/12L$ radians. Similarly, at the end of the second $\frac{1}{100}$ sec. the angle P_2 has a value of $F_2/12L$ radians and therefore the angle traversed during the second $\frac{1}{100}$ sec. is $(P_2 - P_1)$ which equals $(F_2 - F_1)/12L$ radians. Thus the average angular velocity of



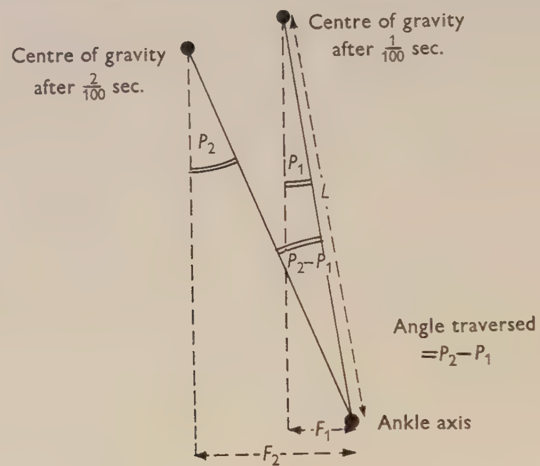
Text-fig. 7. Segment of kymogram of body sway and the line B enlarged and traced on to 1 in. by $\frac{1}{10}$ in. graph paper. Method of calibration.

Table 1. *Method of calculation and tabulation of the values of the terms MLQ and $I_a B/g$ at intervals of the order of $\frac{1}{100}$ sec.*

1	2	3	4	5	6
Time in $\frac{1}{100}$ sec.	Distance of centre of gravity in front of ankle axis by the magnification (X)	$MLQ=\frac{FX.M}{12X}$	Angular velocity of centre of gravity about the ankle axis	Angular acceleration of centre of gravity about ankle axis (B)	$\frac{I_a B}{g}$
1	F_1X	$\frac{MF_1}{12}$			
2	F_2X	$\frac{MF_2}{12}$	$\frac{100(F_2-F_1)}{12L}$	$\frac{100^2(F_3-2F_2+F_1)}{12L}$	$\frac{100^2(F_3-2F_2+F_1)\,I_a}{12Lg}$
3	F_3X	$\frac{MF_3}{12}$	$\frac{100(F_3-F_2)}{12L}$	$\frac{100^2(F_4-2F_3+F_2)}{12L}$	$\frac{100^2(F_4-2F_3+F_2)\,I_a}{12Lg}$
4	F_4X	$\frac{MF_4}{12}$	$\frac{100(F_4-F_3)}{12L}$		

the centre of gravity about the ankle axis during that period is $[100\,(F_2-F_1)]/12L$ radians per sec. The average angular velocity during successive periods of $\frac{1}{100}$ sec. can therefore be calculated and tabulated as in column 4, Table 1.

Angular acceleration is the rate of change of angular velocity and is expressed in radians per sec. per sec. Thus the angular acceleration (B) of the centre of gravity



Text-fig. 8. The angle traversed by the centre of gravity about the ankle axis during $\frac{1}{100}$ sec.

around the ankle axis between the middle of the second $\frac{1}{100}$ sec. and the middle of the third $\frac{1}{100}$ sec. is

$$\frac{\dfrac{100\,(F_3-F_2)}{12L}-\dfrac{100\,(F_2-F_1)}{12L}}{\frac{1}{100}}=\frac{100^2\,(F_3-2F_2+F_1)}{12L}\ \text{rad./sec.}^2.$$

In this way the angular acceleration during successive periods of $\frac{1}{100}$ sec. can be calculated and tabulated as in column 5, Table 1. Thereafter the term I_aB/g can be calculated for the same intervals.

In the equation

$$S=MLQ+\frac{I_aB}{g}-R$$

all quantities are now known except S, the plantar-flexing torque exerted at both ankle joints by the postural activity in the posterior crural muscles of both lower limbs. The value of this factor can now be calculated at intervals of $\frac{1}{100}$ sec.

RESULTS

(1) *The moment of inertia of the body round the ankle axis* (I_a)

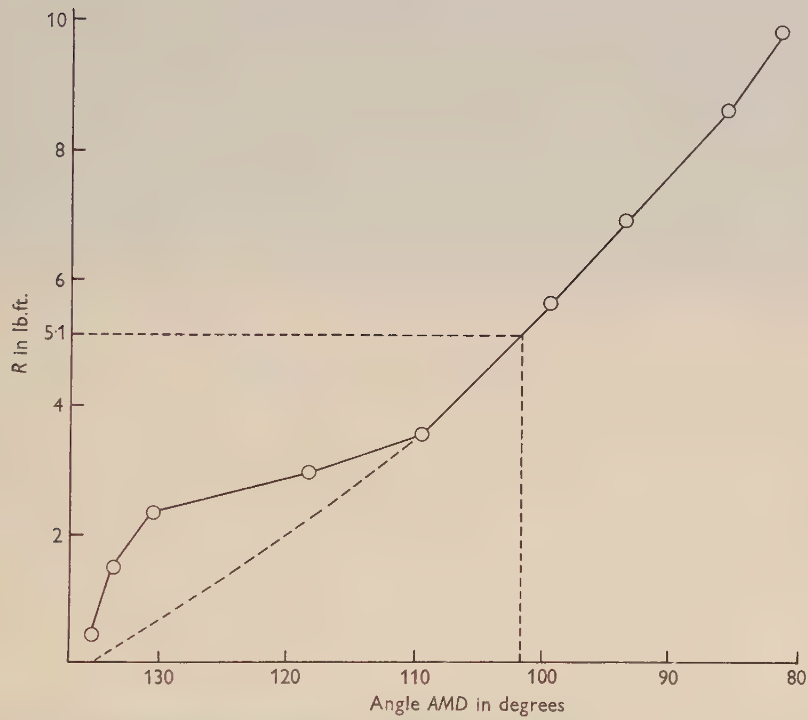
The general formula for the moment of inertia is Mr^2, where r is the radius of gyration which can be defined for the purpose of this investigation as the average distance of all particles in the body from the axis of rotation. It will therefore be apparent that the value of the moment of inertia is closely connected with the body build of the subject, being greater in the tall or heavy than in the short or light.

One example of the determination is given below. In this subject the mass (M) was 189 lb., the distance (D) from suspension to centre of gravity was 7·04 ft., the distance (L) from the centre of gravity to the ankle axis was 2·9 ft., and the time of oscillation (t) was 3 sec. Thus the moment of inertia about the suspension is

$$I = \frac{t^2\,MgD}{4\pi^2},$$

$$= \frac{9 \times 189 \times 32{\cdot}2 \times 7{\cdot}04}{4\pi^2},$$

$$= 9781 \text{ lb.ft.}^2.$$



Text-fig. 9. The relationship of the passive tension in the tissues of the posterior crural regions of both limbs (R) to the angulation of the ankle joint ($\angle AMD$).

By the theorem of parallel axes (p. 547) the moment of inertia around the ankle axis is

$$I_a = I + M\,(L^2 - D^2),$$

$$= 9781 + 189\,(2{\cdot}9^2 - 7{\cdot}04^2),$$

$$= 1994 \text{ lb.ft.}^2.$$

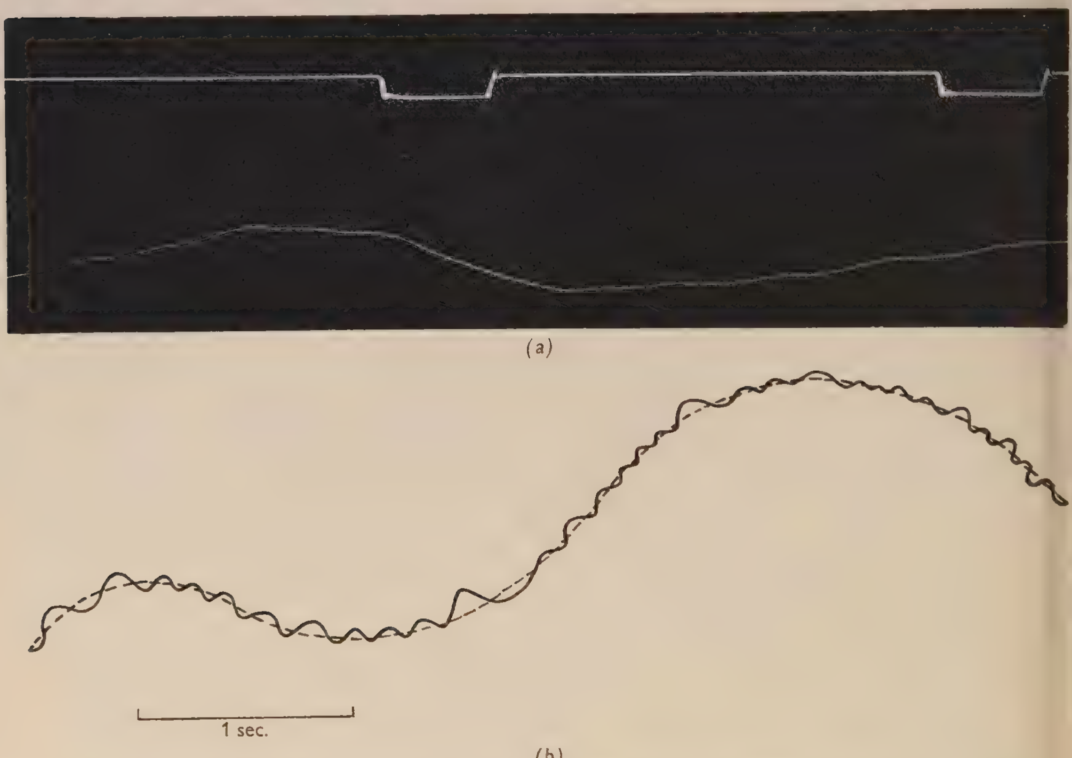
(2) *The passive tension in the tissues of the posterior crural regions of both limbs* (R)

The relationship of (R) to the angulation of the ankle joint ($\angle AMD$ in Text-fig. 3) varied very little in the subjects examined; that shown in Text-fig. 9 is a typical example. That part of the graph associated with dorsi-flexion (110–80°) is almost

linear, whereas that part associated with plantar-flexion (110–135°) shows a distinct 'hump'. This deviation is the result of the approximation described on p. 549 by which the factor Wh is ignored in the equation

$$R/2 = K.DM.\sin\angle CDM - Wh.$$

It is apparent that the approximation does not appreciably affect the values of R for angulations of the ankle joint less than 110°. In the subject to which the data in Text-fig. 9 apply the single value of the angulation of the ankle joint during



Text-fig. 10. (*a*) Enlarged segment of the kymogram in Text-fig. 5*a*. (*b*) The primary and secondary waves of the kymogram represented diagrammatically by the interrupted and solid lines respectively.

standing which was determined was 102°, and the corresponding value of (R) was therefore 5·1 lb.ft.

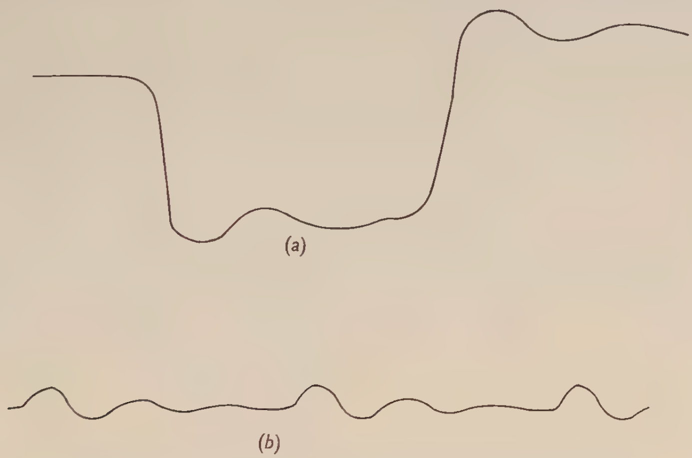
(3) *The values of the terms MLQ and I_aB/g at intervals of the order of $\frac{1}{100}$ sec.*

The kymogram reproduced in Text-fig. 5 indicates the periodic sagittal displacements of the centre of gravity in the standing subject as a series of waves. However, when the kymogram is examined at a higher magnification (Text-fig. 10*a*) it becomes evident that superimposed on these large primary waves is a series of much smaller secondary waves so that the tracing follows the general form indicated diagrammatically in Text-fig. 10*b*.

Both primary and secondary waves are irregular in amplitude and frequency; the average frequency of the former is about 18/min. and that of the latter about 8/sec.

It is certain that the large primary waves of the kymograph tracing accurately represent corresponding displacements of the centre of gravity of the body, but before the smaller secondary waves can be regarded as arising in the same way, the possibility of artefact must be excluded.

Artefacts of such form might arise through vibration either in the kymograph drum or in the magnifying mechanism. Vibration in the drum is excluded by the absence of any ripples on the tracing when the magnifying arm is attached to a fixed point. Vibration in the magnifying mechanism might be evoked by either of two distinct factors. Thus, if there were a sudden large alteration in the angular velocity of the swaying body, this would be indicated by a correspondingly sudden displacement of the magnifying arm. The momentum of the magnifying arm might



Text-fig. 11. The form of two types of vibration waves.

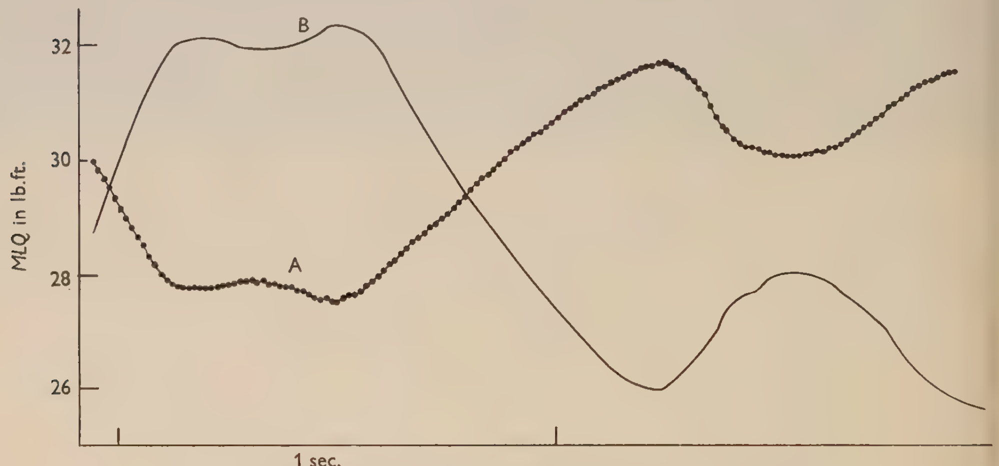
then be sufficient to cause a temporary elastic distortion of the whole mechanism and initiate a series of vibrations. In such circumstances the ripple would be restricted to those parts of the kymogram which immediately follow steep upward or downward deflexions, and would show a regular frequency and progressively diminishing amplitude as indicated diagrammatically in Text-fig. 11*a*.

Secondly, vibration in the magnifying mechanism might be evoked by a continuously repeated series of sudden impulses on the centre of gravity of the body such as those imparted by the normal pulse. The ripple would then follow a pattern of repeated sequences of waves, each sequence having a uniform frequency and progressively diminishing amplitude as indicated in Text-fig. 11*b*.

Thus any artefact would be characterized by a certain regularity of pattern. The fact, therefore, that in the kymogram of body sway the secondary wave series follows no recognizable pattern, indicates, of itself, that these waves do not arise as artefacts. Moreover, this is confirmed by the fact that the form of the secondary wave series can be shown to be independent of the vibration time of the magnifying mechanism.

It is considered, therefore, that both the primary and secondary waves indicate proportionate displacements of the centre of gravity during standing, and that both must consequently be taken into consideration in the calculation of the terms MLQ and $I_a B/g$.

The values of the term MLQ calculated at intervals of $\frac{1}{140}$ sec., and the segment of the kymogram in Text-fig. 5*a* from which they were assessed, are shown in Text-fig. 12. The value of the term shows a continual fluctuation which is synchronous with and proportional to the displacements of the kymogram of body sway; the fluctuations are thus almost entirely dependent on the primary kymogram waves and are influenced very little by the smaller secondary waves. During a stance of average duration, in a subject of average physique, the fluctuations occur within a range such as 26–34 lb.ft. The amplitude of the fluctuations varies considerably but has an average value of about 2 lb.ft.



Text-fig. 12. The line A indicates the values of MLQ calculated at intervals of $\frac{1}{140}$ sec.; the line B is the corresponding segment of the kymogram of body sway.

Text-fig. 13 shows the values of the term $I_a B/g$ at intervals of $\frac{1}{84}$ sec. This factor undergoes a rapid and continuous fluctuation between negative and positive values at rapid and irregular intervals of about $\frac{1}{10}$ sec. Close comparison of this graph with the original kymogram shows that these fluctuations are synchronous with the secondary wave series, and are influenced very little by the form of the larger primary waves. During a stance of average duration, in a subject of average physique, the largest fluctuations are between about ± 8 lb.ft. whereas the average fluctuation occurs between $\pm 2{\cdot}5$ lb.ft.

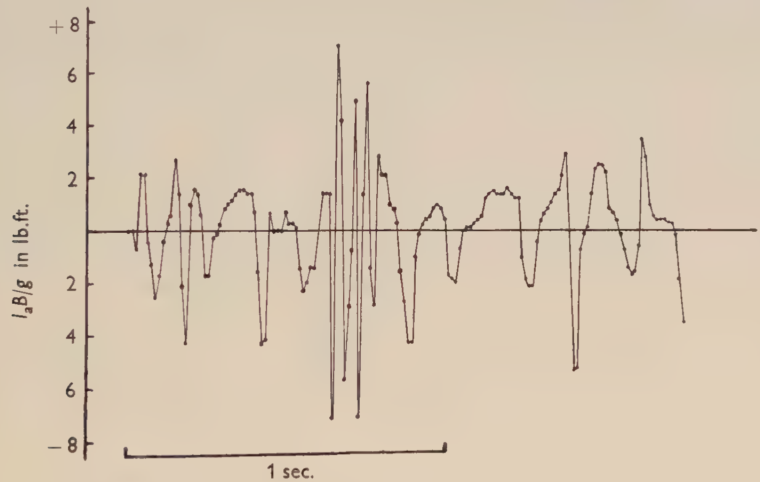
Thus of the two wave series in the kymogram (Text-fig. 10) the primary series are associated with small and comparatively gradual variations in the term MLQ, whereas the secondary series are associated with larger and more rapid changes in the term $I_a B/g$.

(4) *The plantar-flexing torque exerted at the ankle joints by postural activity in the posterior crural muscles of both limbs* (S)

The value of the active plantar-flexing torque operating at both ankle joints (S) is given by the formula

$$S = MLQ + \frac{I_a B}{g} - R \text{ lb.ft.}$$

Consideration of the practically constant value of R, and of the types of fluctuation which the values of MLQ and I_aB/g undergo, makes it clear that the value of S will fluctuate, in the manner of the term I_aB/g, about a mean which itself fluctuates in the manner of the term MLQ. It is apparent that because the factors M, L and I_a are dependent on the body type of the individual the value of S will vary with physique, being larger in tall or heavy subjects than in those who are small or light. However, in a young adult male of average physique it can be said that during a prolonged stance, as has been noted on p. 558, the value of MLQ varies in the range 26–34 lb.ft. and therefore since R is equal to about 5 lb.ft. and I_aB/g fluctuates between maximum and minimum limits of ± 8 lb.ft., the value of S will vary between maximum and minimum values of about 13 and 37 lb.ft. On the other hand, during any short period of stance of about 1 sec. the average fluctuation of MLQ is 2 lb.ft. and that of I_aB/g is $\pm 2{\cdot}5$ lb.ft. and therefore the average fluctuation of S is over a range of about 7 lb.ft. somewhere between the limits 13–37 lb.ft.

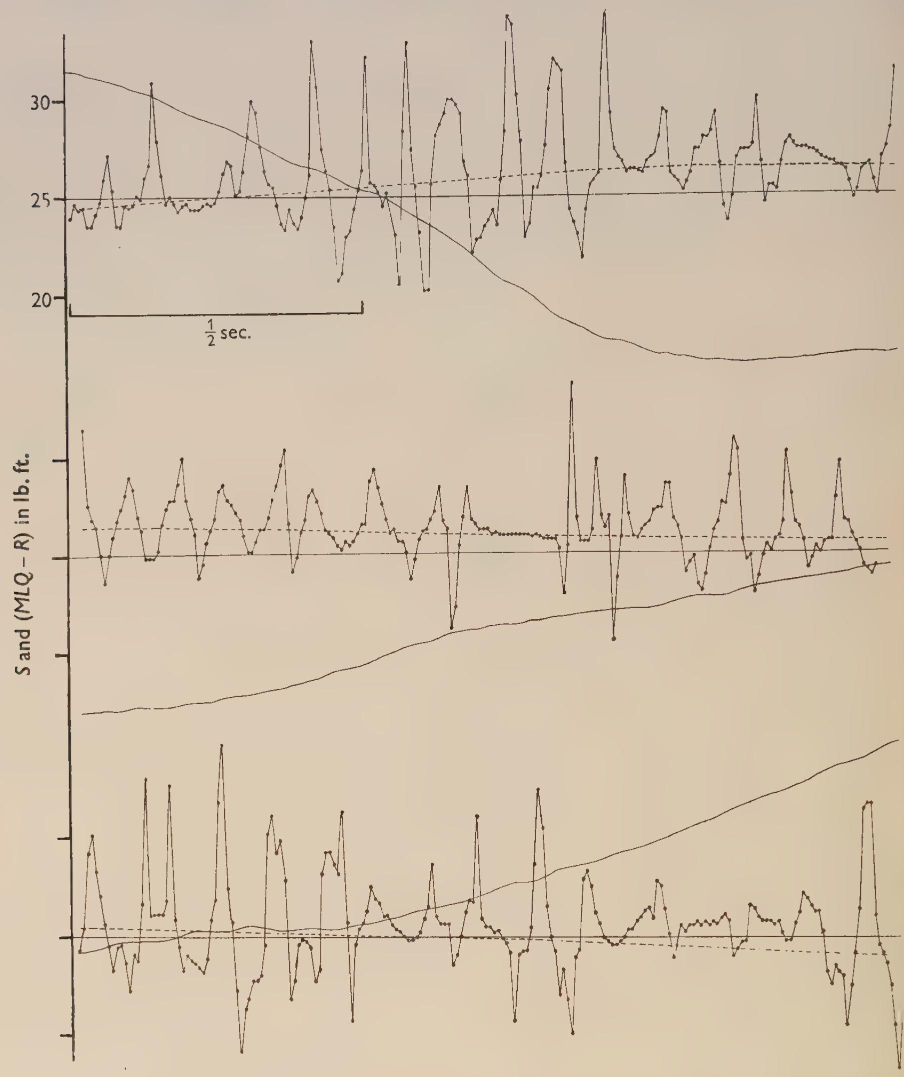


Text-fig. 13. The value of the term I_aB/g at intervals of $\frac{1}{84}$ sec.

Text-fig. 14 shows the values of S calculated at intervals of $\frac{1}{140}$ sec. over a period of about 4 sec. On the same graph variations of the factor ($MLQ - R$) over the same period are indicated by the interrupted line, and the corresponding part of the kymogram in arbitrary units is shown by the continuous line.

DISCUSSION

During standing the body oscillates antero-posteriorly over the foot, but although the centre of gravity consequently bears a variable relationship to the ankle axis it is always anterior to it. Thus body weight exerts at the ankle joint a dorsi-flexing torque which is constant in direction but variable in magnitude. The upright



Text-fig. 14. The values of S at intervals of $\frac{1}{140}$ sec. in a subject of average physique. The term ($MLQ-R$) is indicated by the interrupted line and the corresponding part of the kymogram of body sway by the continuous line.

posture is achieved by a plantar-flexing torque which continually counters the effect of body weight, although it exactly equals and neutralizes it only at the anterior and posterior extremes of each oscillation of the body. Between these extremes the difference between the plantar-flexing and dorsi-flexing torques determines the

direction and magnitude of the angular acceleration of the body around the ankle axis.

The plantar-flexing torque is the sum of that exerted by the active contraction of the posterior crural muscles and that exerted by the passive tension in all the tissues of the posterior crural region ($T = S + R$).

In a young adult of average physique the passive torque in each lower limb ($R/2$) has an approximately constant value of about 2·5 lb.ft. On the other hand, the value of the active torque in each lower limb ($S/2$) fluctuates about ten times per sec. about a mean which itself fluctuates synchronously with the gross swaying of the body at a rate of about twenty times per min. Over a period of a few minutes it may vary within limits of about 6 and 18 lb.ft. while over a shorter period such as 1 sec. it usually fluctuates to an extent of 3 or 4 lb.ft. in the range 6–18 lb.ft. Thus despite the variable nature of the active torque it is always at least twice as great as the passive torque.

In a previous investigation (Smith, 1956) it was noted that the flexing torque which stabilizes the knee joint during symmetrical standing is similarly derived from active and passive sources, but in very different proportions, the active contribution being 30 % and the passive contribution 70 %. The smaller passive contribution at the ankle joint is dependent on a smaller passive tension in the relative extra-articular tissues. This in turn is due in part to the fact that, whereas in standing the knee joint is close to full extension, the ankle joint in similar circumstances is some 20° or so short of full dorsi-flexion. Another reason lies in the nature of the limiting articular mechanism at the two joints. That limiting extension at the knee joint operates over the terminal 10° or so of movement and consequently contributes to the passive force stabilizing the knee joint in standing. That limiting dorsi-flexion at the ankle joint, on the other hand, as can be shown in an osteo-ligamentous preparation of a recently amputated limb, operates over only the terminal 3° of movement and thus contributes nothing to stability during standing.

The exact location of the postural muscle activity which gives rise to the active plantar-flexing torque is not known with certainty, and consequently the torque cannot be accurately expressed in terms of muscle tension. However, despite the lack of certainty, there is some evidence (Smith, 1954) which suggests that a very large measure, if not all, of the activity is located in the triceps surae. If this distribution is accepted, it follows that the muscle tension is transmitted almost entirely through the tendo calcaneus, and that its value can be calculated by dividing the active plantar-flexing torque by the distance between the tendon and the ankle axis. The latter distance has been measured in fifteen subjects of average physique and has been found to vary from 0·08 to 0·12 ft. with an average value of about 0·1 ft. Thus the muscle tension developed in the triceps surae of each limb during standing in one of average physique varies between 60 and 180 lb. during a stance of average duration, and to an extent of about 35 lb. in that range over any period of a second or so.

This tension is considerably greater than that suggested by Joseph & Nightingale (1952). They suggested that in a subject of 140 lb. body weight the tissue tension, both active and passive, had a value of about 35 lb. However, their figure depended on the distance between the tendo calcaneus and the ankle axis being 8 cm., whereas,

as noted above, the present investigation shows that the distance has an average value of 0·1 ft. or 3 cm.

The absence of fatigue in the triceps surae during standing, would seem to be associated with two factors. First, the muscle tension, high as it is, is only a small fraction of that commonly developed during normal activity. Thus Elftman (1939) has estimated that at a moderate rate of walking each triceps surae develops a tension at each step of about 600 lb. And secondly, the act of standing is essentially periodic in nature (Smith, 1953), periods of immobility, of an average duration of 30 sec., being separated by brief phases of movement in which the position of the body is shifted. Thus throughout standing the triceps is momentarily relieved of its burden of activity at frequent intervals.

The large amplitude of fluctuation in the value of the muscle tension in the triceps surae is also of interest. It might appear that fluctuations in muscle tension in the two limbs to an extent of 70 lb. at a frequency of about 10 cyc./sec. is incompatible with the very small variations in the position of the body which result. However, the displacement of a body does not depend only on the forces applied to it; it depends also on the duration of the force and on a third factor, which in the case of linear motion is the mass of the body and in the case of angular motion is the moment of inertia. Thus, even a large force opposing for a brief period the movement of a heavy flywheel would cause little change in its angular velocity because of the large moment of inertia of the flywheel and the short duration of the opposing force. Similarly, because of the large moment of inertia of the body about the ankle axis and the high frequency of the fluctuations of muscle tension, these large changes in the tension in the posterior crural muscles cause little displacement of the body as a whole.

As regards the cause of the fluctuations in muscle tension it seems apparent that they are the result of two factors, namely, an incomplete tetanus of the motor units involved and a measure of synchronization in the firing of these units. Such an explanation is fully in keeping with the observations of Denny-Brown & Nevin (1941) and Denny-Brown (1949), who noted that postural activity of muscle differs from that associated with voluntary movement in two respects. It increases by recruitment of units all firing at a low maximum rate of 5–10/sec. in contrast to the increase of willed effort both by recruitment and by an increase in the rate of firing of previously active units. Secondly, it is characterized by a periodicity in the intensity of the discharge of action potentials and consequently by a 'clonic' type of electromyogram; this is in contrast to the typically asynchronous discharge and continuous electromyogram of voluntary movement.

It is difficult to reconcile the results of this mechanical estimation of the tension resulting from postural activity in the posterior crural muscles during standing with those of the many electromyographic examinations published during recent years. These results have varied considerably. Ralston & Libet (1953), reporting the results of the biomechanics group at the University of California, maintain that no activity occurs in the calf muscles during standing, except during excessive swaying of the body. On the other hand, Kelton & Wright (1949), Floyd & Silver (1950) and Smith (1954) have all observed a mild degree of activity which was intermittent in nature and synchronous with the antero-posterior swaying of the body. And

thirdly, there is a large body of opinion, of which the works of Hoefer (1941), Seyffarth (1940), Weddell, Feinstein & Pattle (1944), Joseph & Nightingale (1952) and Joseph, Nightingale & Williams (1955) are representative, which considers that activity is continuous and of more or less uniform intensity throughout standing.

Consideration of the variations in muscle tension during standing described above suggests that the appearance of an electromyogram might well vary with its duration. Thus an electromyogram of short duration might be expected to show a continuous discharge of practically uniform amplitude, whereas a longer recording should show a discharge which, although continuous, fluctuated considerably in amplitude. It therefore seems reasonable to infer that the recordings quoted above which show an intermittent activity indicate either an instrument of insufficient sensitivity, as Joseph & Nightingale (1952) have suggested, or an unconscious exaggeration of body sway on the part of the subject—an exaggeration which can readily occur if the subject suspects the purpose of the experiments. On the other hand, those recordings which show no significant variation in amplitude with body sway may simply be too short to show such a variation; variations in amplitude synchronous with body sway are very difficult to appreciate on the fluorescent screen and are only readily apparent when recordings of over about 10 sec. are examined.

Quite apart from the exact pattern of the muscular discharge in standing, the results of the present investigation seem to have a bearing of some importance on the general use of surface electromyography. In their examination of the posterior crural muscles in standing, Joseph *et al.* (1955), using a very sensitive and well screened instrument, obtained deflexions of the order of 40 μV. above the background disturbance. The present results show that these deflexions represent the electrical activity associated with a comparatively high muscle tension of the order of 120 lb. It therefore seems questionable whether surface electromyography in its present form is to be regarded as an adequate method for determining inactivity or assessing minor degrees of activity in muscle—purposes for which it has been frequently employed during the last few years.

SUMMARY

1. During standing gravity constantly tends to carry the body forwards around the axis of rotation of the ankle joint. This dorsi-flexing force is resisted, and the upright posture is maintained by active and passive forces tending to cause plantar-flexion at the ankle.

2. Methods are described by which these active and passive forces can be assessed.

3. The active force is the result of a postural contraction which is located in large measure in the triceps surae. The magnitude of the force fluctuates continually at a rate of about 10 cyc./sec. between high and low values in the range 80/160 lb.

4. The passive force is the result of tension in passive extra-articular tissues in the posterior crural region. The plantar-flexing torque which it exerts at the ankle joint remains approximately constant throughout stance and has a value of about 2·5 lb.ft.

5. The results of this investigation are correlated with those of the numerous electromyographic studies of the posterior crural muscles in standing which have been reported in recent years.

I wish to thank Prof. R. Walmsley for his interest and advice. I am also very indebted to Prof. A. E. Ritchie for his criticisms and to Dr Bijl of the Department of Natural Philosophy for his advice on mechanics.

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REVIEWS

Bone Structure and Metabolism. Ciba Foundation on Bone Structure and Metabolism. Edited for the Ciba Foundation by G. E. W. WOLSTENHOLME, O.B.E., M.A., B.Ch., and CECILIA M. O'CONNOR, B.Sc. (Pp. 299+xii; illustrated. 45*s*.) London: J. and A. Churchill. 1956.

This symposium follows a familiar pattern. Leading workers in the field of experimental osteology were invited to give papers, and these, together with full verbatim reports of the discussions after each paper, are published. In all there are twenty papers with twenty-eight participants. To begin with, there are papers on bone structure and composition, then a series of metabolic studies of bone growth and repair, accounts of recent work on vitamin D and the parathyroids, and, finally, reports on natural and experimental bone disease. The quality is very uneven: some papers are excellent, one or two are incomprehensible. In some ways the discussions are the most interesting and valuable parts of the book: some critical and illuminating, others irrelevant or exhibitionist, but all showing current trends of thought in the field of bone research.

The symposium as a whole reveals the growing co-operation and understanding between histologists and biochemists, cytochemistry acting as the link. Osteoblasts and chondroblasts seem to be generally accepted as secretory cells, and the approach to most bone problems is dynamic. The value of newer techniques is strongly brought out, especially the use of radio-isotopes (employed in almost half of the work reported), electron microscopy, X-ray microscopy, X-ray and electronic diffraction, and interferometry. The achievements and future possibilities of isotope studies for cytological, growth and metabolic problems alike are probably the most exciting aspects of modern bone research.

There is hardly any discussion of the role of alkaline phosphatase, suggesting that this ubiquitous enzyme is in eclipse. One gets the impression also that the vascular anatomy and physiology of bone have been neglected and deserve intensive study.

All things considered, the symposium is necessary reading for workers in the osteological field, and desirable reading for biologists generally. J. J. PRITCHARD

Primates. Part 3—Platyrrhini (Families Hapalidae and Callimiconidae). By W. C. OSMAN HILL. (Pp. xix+354; 27 plates and 102 text-figures. 90*s*.) Edinburgh University Press.

Having been asked to write a notice of this book, we welcome the opportunity once more to express our gratitude to the author for preparing such a fine series of monographs on the Primates. It would hardly be possible to exaggerate their importance as up-to-date works of reference. The present volume deals with the marmosets which Dr Hill groups in two separate families, but he notes in his preface that, even while the book was in the press (and too late for revision), he had arrived at the conclusion from new studies that, after all, *Callimico* is not deserving of a familial distinction from the Hapalidae. Fluctuations in classifications of this sort are bound to occur from time to time as more detailed knowledge accrues, and whether a disputed group should be categorized as a family or a sub-family may not be of very great moment. But difficulties and confusions are bound to arise if well-recognized *major* taxonomic categories are re-arranged or re-named, and one cannot but regret that this has been thought desirable in what is intended to be a standard work of reference. So far as we are aware, the classification of the Primates which Dr Hill adopts is not in use by any other authority. He has allowed himself to be persuaded by arguments advanced forty years ago by Pocock, arguments which seem to us unreasonable. The term Anthropoidea for the suborder containing monkeys, apes, and hominids has been generally

accepted for almost a hundred years, but Pocock wished to dispose of it because (1) a marmoset can hardly be called 'anthropoid', and (2) it so happens that English scientists refer colloquially to the 'anthropomorphous apes' as 'anthropoid apes', or even (carelessly) as 'anthropoids'. He therefore proposed the substitution of the term 'Pithecoidea' for the suborder, for the reason, it seems, that 'a marmoset and man are alike "pithecoid"'. Although Dr Hill appears to accept this curious argument, he begins his general account of the suborder by the statement: 'All the Pithecoidea agree in their generally manlike form.' But since the term Anthropoidea was originally proposed by Mivart in 1864 to give expression to just that likeness, why should it now be changed? Pocock's other argument against this long accepted term is surely an extreme example of the proverbial parochialism of the Englishman—that, because of a loose terminology sometimes used by *English* scientists, therefore an *international* nomenclature must be changed! Another comment which might be made refers to Dr Hill's taxonomic definitions of the major categories of the Primates, for here he follows the custom of the old naturalists of former days by defining them in static terms of those representatives which exist to-day rather than in terms of trends in the main adaptive radiations of which these representatives happen to be the few surviving and terminal products. Thus in the definition of the 'Pithecoidea' it is stated that they differ from the Tarsioidea by the relatively small eyes. From the modern *Tarsius*, yes; but would the distinction apply to all the fossil tarsioids which Dr Hill lists in his previous volume? It will also be disconcerting to the reader to learn that one of the diagnostic characters of the 'Pithecoidea' is that the tympanic chamber is without inferior inflation, and later to learn that in the whole group of the Platyrrhini (Ceboidea) the tympanic chamber still retains the primitive inflation of the bulla.

It seems important to draw attention to these taxonomic difficulties, if only because the diversity of classifications of the Primates proposed by individual scientists has in the past been one of the main causes of confusion and misunderstanding in discussions on their phylogenetic relationships. The family of the Equidae, which includes the whole evolutionary sequence from the little *Hyracotherium* of Eocene times, can no longer be defined as Cuvier once defined his equivalent of the horse family, that is, by characters such as a single complete digit on each foot and the complicated pattern of the cheek teeth. No more can the families of the Primates be defined in terms of the surviving terminal products of evolutionary sequences. But, even though the taxonomic sections of Dr Hill's monographs will certainly arouse some regret and criticism, we remain firm in our admiration for this comprehensive encyclopaedia of the order of Primates, to a knowledge of whose anatomy the author has himself contributed so much original work.

W. E. LE GROS CLARK

PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1956

The Annual General Meeting of the Society for the Session 1956–7 was held on Friday, 30 November and the morning of Saturday, 1 December 1956 in the Department of Anatomy, St Mary's Hospital Medical School, London, W. 2. The President (Prof. R. D. Lockhart) was in the Chair for the Friday sessions. The communications on the Saturday were arranged in two sessions running concurrently, with the newly elected President, Prof. W. J. Hamilton, and Dr G. Weddell acting as chairmen.

The following are the authors' abstracts of the papers presented.

A quantitative study of transneuronal atrophy in the human lateral geniculate body. By F. Goldby. *St Mary's Hospital Medical School, London*

The specimens described consist of the lateral geniculate bodies from a man who lost his left eye about 40 years before death. The sizes of cells in both normal and atrophic laminae have been estimated by planimetric measurements in sample groups of cells, with the following results.

The mean projection areas for the large cells in laminae 1 and 2 (normal) are $443 \pm 103\,\mu^2$ and for laminae 3–6 (normal) $296 \pm 67\,\mu^2$. When atrophic these cells become reduced in area by about 32 % (laminae 1 and 2) and by about 48 % (laminae 3–6). This confirms the impression from inspection that the large cells are less sensitive to loss of retinal connexions than the smaller cells of the deeper laminae.

It is found also that cell density in the atrophic laminae is about the same as in the normal laminae, although the former have shrunk by about 50 %. It appears that in addition to cell atrophy, there must have been cell destruction up to about 50 % of the original number in the affected laminae.

As expected, the atrophy which results from loss of retinal connexions is much greater in man than that which was found in the cat by Cook, Walker & Barr (1951), where atrophic cells were reduced by about 25 % in sectional area. Their longest survival period was 10 months, so that longer-term experiments in animals are necessary before the difference can be taken as firmly established.

Some effects of temperature on the rate of degeneration and regeneration in a peripheral nerve. By H. J. Gamble, F. Goldby and G. M. R. Smith. *St Mary's Hospital Medical School, London*

Previous experiments on reptiles (e.g. Armstrong, 1951) have shown that processes involved in axonal and terminal degeneration in the central nervous system of a reptile may be greatly accelerated by raising the environmental temperature. Further experiments have been carried out to investigate this temperature effect more precisely, and for this purpose a nerve in *Lacerta viridis* corresponding to the lateral popliteal nerve has been used.

The effects of cutting or crushing this nerve in animals subsequently kept for different periods in temperatures which did not vary more than a few degrees from 35°, 22° and 13°C., respectively, have been studied. The cloacal temperatures of the animals (measured with a thermocouple) were found to approximate very closely to that of the surrounding air.

It was found that at 13°C. the degenerative changes after 21 days were approximately the same as those after 3 days in an animal kept at 35°C., animals at 22°C. showing an

intermediate condition. The details of the axonal and sheath changes were illustrated and discussed.

Evidence has also been obtained that regeneration after crushing is similarly accelerated at higher as compared with lower temperatures.

Development of the interorbital septum in chick embryos. By A. d'A. Bellairs. *St Mary's Hospital Medical School, London*

The interorbital septum in the chick embryo is developed from three elements: the trabeculae and orbital cartilages, which are paired, and a median tract of tissue known as the intertrabecula. Though never completely separate from each other these elements can be distinguished in procartilaginous stages of skull development. Later they merge with each other to form the cartilaginous interorbital septum of the formed chondrocranium.

In the adult fowl the olfactory nerves reach the nasal sacs by passing through the orbits, where they groove the interorbital septum. This relationship is reached as the result of complex changes in the morphology of the septum and adjacent cartilages, including regression, and an apparent rotation of the anterior orbital cartilages. It is not affected by removal of the eye prior to the stage of skull formation.

Cautery experiments on the development of the acoustico-facialis ganglion of *Lampetra*. By Alan Fisk. *St Mary's Hospital Medical School, London*

It has been believed for many years that the acoustico-facialis ganglion of *Lampetra* originates from both neural crest and placodes. Recently it has been stated, from the study of a series of normal embryos, that the cranial part of the ear placode forms the neuromast ganglia (which are extracapsular), while the ventral part of the ear placode contributes the acoustic components, and the neural crest contributes to the intracapsular facial ganglion (i.e. to the somatic sensory and possibly also the visceral sensory components).

A series of experiments is described in which portions of the superficial ectoderm, including dorsolateral placodes, were destroyed by cautery. The young ammocoetes which developed from these treated embryos showed deficiencies which support a number of the earlier findings based on the study of normal embryos. Experiments were not carried out on the first epibranchial placode.

Cautery was performed with a fine tungsten needle, and a small innovation in technique, the addition of chicken-egg white to the suspending fluid of the embryo, made possible the exposure of the embryo for this purpose; the exposure of young *Lampetra* embryos on the surface of water is impossible ordinarily because of surface-tension effects.

Anophthalmia and the optic system in the chick embryo. By R. M. Collister. *University College, London*

Incubation of hens' eggs in an oxygen-poor atmosphere, 12–14 %, has produced unilateral anophthalmia as reported in the literature. Serial sections were cut of two such abnormal embryos; one at 7 days and one at 12 days incubation. The optic systems were examined microscopically. In the embryo of 7 days the left eye was represented by a rudimentary optic vesicle deeply placed in the mesoderm. This had become rotated through 180° from its normal relationship with the diencephalon, as had the local condensation of the mesoderm. The optic nerve was absent on this side. The development of the optic tecta and the thalami was normal on both sides. On the right side the eye and its related structures had developed normally. In the 12-day embryo not even a rudimentary eye was found on the abnormal side, the left, and the optic nerve on this side was also absent. The optic tecta and the thalami had developed without apparent abnormality and were equally developed on both sides. The findings strongly indicate that the optic tectum develops independently of the eye until the twelfth day.

The development of the human basal ganglia. By W. Hewitt.
St Thomas's Hospital Medical School, London

The usual description of the development of the basal ganglia is that they are formed from two parallel ridges which appear in the floor of the cavity of the cerebral hemisphere. While this is true in the early stages of development, a third elevation later appears between the original ridges, and the caudate nucleus is ultimately formed by the fusion of all three elements. The lentiform nucleus appears to be formed from cells lying deep to the ridges and continuous with them. In coronal section, the whole mass of the developing basal ganglia at first resembles a cap of cells with its hilum directed towards the site of the future cerebral peduncle. In the early stages of development the internal capsule does not extend out to the cerebral cortex, but merely connects the hilum of the cap of cells to the site of the future cerebral peduncle. As development proceeds the internal capsule develops from behind forwards and extends out to the cerebral cortex and cuts through the cap of cells separating the caudate and lentiform nuclei from one another except where they remain connected in adult life.

Ascending fibres in the medial forebrain bundle of the rat.
By R. W. Guillery. *University College, London*

Lesions have been placed in the hypothalamus and midbrain of albino rats and the subsequent fibre degeneration has been studied by the method of Nauta and Gygax. After a lesion in the premamillary hypothalamus two groups of ascending fibres can be traced into the septum. One, composed of relatively fine fibres, ends in the lateral septal nucleus, the bed nucleus of the anterior commissure and the dorsal part of the nucleus accumbens. The other, composed of coarser fibres, ends in the nucleus of the diagonal band, the medial septal nucleus and the ventral part of the nucleus accumbens; a few of these fibres can also be traced towards the hippocampus. Whereas the fine lateral component does not degenerate after lesions in the supramamillary region and midbrain the coarse medial component does. It is concluded that two distinct ascending bundles reach the lateral and medial septal regions via the medial forebrain bundle, a fine-fibred hypothalamoseptal bundle and a coarse-fibred mesencephalo-septal bundle.

Median eyes, their innervation and its bearing on the ancestry of the pineal. By C. C. D. Shute. *University of Cambridge*

Because of difficulties inherent in the current view that the vertebrate epiphysis consists of two distinct, originally paired evaginations of the diencephalic roof, the pineal and parapineal organs have been restudied with reference to their origin and nerve supply.

It is concluded that in all cases the pineal complex is derived from a single mesencephalic diverticulum, the extremity of which gives rise to the parietal eye of reptiles and to the pineal eye of lampreys, which are therefore homologous. The median eye is innervated primitively by the pineal nerve, which in *Lampetra* does not connect with the diencephalon, but traverses the original pineal stalk to enter the midbrain by the posterior commissure. The parapineal of lampreys is not itself eye-like in structure. It originates as a ventral diverticulum from the pineal eye, from which it becomes detached at an early ammocoete stage. The vesicle so formed establishes a secondary neural connexion with the left habenular nucleus. A similar parapineal diverticulum occurs as a vestige in ganoid fishes and probably also in some early lizard embryos. In *Lacerta*, *Anguis* and *Anniella* the nerve of the parietal eye can be traced constantly to the left habenular nucleus. It is suggested that in reptiles the median eye has lost its primitive mesencephalic innervation, but retains a habenular connexion through the parapineal vestige—a pineal eye, that is, supplied by a parapineal nerve. In the chameleon *Microsaura*, the nerve enters the habenular commissure in the midline.

In mice and rats habenular commissural fibres loop into or cross behind the pineal; there is little innervation from the posterior commissure proper. The nerve which supplies the outlying distal portion of the organ probably arises from ganglionic cells in the pineal stalk and corresponds to the nervus conarii of other mammals.

The cellular structure of rabbit cardiac muscle, as observed with the electron microscope. By A. R. Muir. *University of Edinburgh*

The intercalated disc is bisected by two adjacent cell membranes, the myofilaments of each cell terminating at the cell membrane in dense plaques. In the adult, the cell membranes are folded, so that, with the adjacent plaques, the thickness of the disc is about $1\,\mu$.

Sixteen stages of pre- and post-natal development have been studied. At all ages when a myofibrillar axis is crossed by a pair of cell membranes, a structure similar to the adult intercalated disc is observed. The intercalated discs always occur in series with the Z membranes of the myofibrils in each cell. In the younger hearts, the folding of the cell membrane is absent, so that the thickness of the discs is only about $0{\cdot}2\,\mu$, and therefore they cannot be distinguished from Z membranes by the light microscope.

The embryonic cardiac muscle cell is uninucleated and spindle-shaped, while in the adult the territory between intercalated discs contains more than one nucleus and is larger than the embryonic cell. The significance of these observations in relation to the growth of the heart and the transmission of the impulse for contraction is discussed.

Preliminary findings in an electron microscopic investigation of the rat parathyroid. By J. D. Lever. *University of Cambridge*

The rat parathyroid contains a single parenchymal cell type within which are observed variations in the overall electron density of the cells. Some cells contain numbers of granular-walled saccular elements of the endoplasmic reticulum (ergastoplasm) and many of their mitochondria are swollen and disorganized; others are more electron-dense with few cytoplasmic sacs and more intact mitochondria. The morphology and distribution of the mitochondria, the Golgi elements and certain lipid bodies is demonstrated; and a relationship is suggested between certain elements of the endoplasmic reticulum and the nuclear membrane. Throughout the tissue is a system of intercellular spaces bounded by the plasma membranes of adjacent parenchymal cells. These intercellular spaces are in contact with the 'subendothelial' spaces which lie between the blood sinusoid endothelium and the juxtaposed parenchymal cells. Both these space systems have been observed in other endocrine tissues.

Further comments on tissue fluorescence induced by acridine orange R. By J. A. Armstrong. *National Institute for Medical Research, Mill Hill, London*

When thin sections are treated with the fluorochrome Acridine Orange R, within the pH range 3·5–5, they acquire polychromatic fluorescent properties which seem to permit histological differentiation of the nucleic acids (DNA and RNA). Further experiments have been made in an attempt to clarify some of the mechanisms concerned in this phenomenon.

It appears that chemical fixation, although convenient, is not necessary; also that certain impurities present in the acridine compound have no significance. Some features of the dye take-up process, and also attempts to eliminate fluorescence from treated sections, indicate that the two kinds of nucleic acid differ somewhat in the nature of their affinity for Acridine Orange R. From this, and observations on the fluorochroming properties of some other acridine derivatives, it is inferred that the bond with DNA is strong and perhaps of a chemical nature, whilst that with RNA is less stable in some circumstances. This may account for the rather different results which have been reported following *in vivo* administration of amino-acridines.

The reactions of the olfactory receptors to lesions of the olfactory bulb. By W. E. Le Gros Clark and T. P. S. Powell. *University of Oxford*

Ablation of one olfactory bulb is followed in 24 hr. by a massive degeneration of the olfactory receptors of the same side, and in about 48 hr. this process of disintegration reaches a maximum. By 72 hr. it appears to be practically completed and the debris of the affected cells is cleared away by the agency of the supporting cells. Previously recorded experiments by one of the authors presented evidence that, contrary to the opinions of some earlier workers, the degeneration is not the result of an involvement of the ethmoidal vessels supplying the olfactory mucous membrane. However, in view of the rapidity and violence of the degenerative reaction, which in effect is a necrosis of the receptor cells, the question has been re-investigated by repeating and extending these experiments. Destruction of the bulb without direct encroachment on the region of the cribriform plate, and restricted lesions of the dorsal surface of the bulb, produces consistent and confirmatory results, i.e. general or localized degeneration of the olfactory receptors. On the other hand, simple incision of the bulb without interruption of olfactory nerve fibres does not lead to any evident degeneration, nor does complete interruption of the olfactory pathways behind the bulb. Further, not only ablation of the bulb, but also a small focal lesion limited to the accessory bulb, leads to a similar rapid disintegration of the receptors in the vomero-nasal organ, although the latter is not within the vascular territory of the ethmoidal vessels. It is concluded that the degeneration of the olfactory receptors in all these experiments is the direct result of axonal interruption. However, following total bulbar ablation on one or both sides a proportion of the receptors persist for at least 6 months. Several possible explanations of these residual receptors present themselves. For example, they might be intrinsic elements of the olfactory epithelium, or replacements due to a rapid regenerative process, or (as suggested by the electrophysiological observations of Beidler and Tucker, *Science*, **122**, 1955) their axons might be conveyed along branches of the trigeminal nerve. These possibilities appear to be excluded by tracing their axons in silver sections, by the study of a closely graded time series, and by combining bulbar ablation with intracranial section of the fifth nerve. Other possibilities are under investigation.

The thalamostriate projection in the avian brain. By T. P. S. Powell and W. M. Cowan. *University of Oxford*

In view of the recent demonstration of an extensive thalamostriate projection in the mammalian brain an investigation has been made of the thalamic connexions of the highly specialized avian striatum. The retrograde cell degeneration in the thalamus of the pigeon has been studied after varying lesions had been placed in the telencephalon. Following removal of the entire telencephalon the nuclei rotundus and ovoidalis have completely degenerated together with the nuclei of the dorsal group, the nuclei sub- and post-rotundus and the nucleus superficialis parvocellularis. That these nuclei project independently of each other is apparent from the finding that in different experiments individual nuclei selectively undergo degeneration. After smaller lesions involving any of the subdivisions of the striatum other than the paleostriatum, only the dorsolateral nucleus shows changes; the precise area to which this element projects, however, has not been determined. The nuclei rotundus, ovoidalis and dorsomedialis show degeneration only when the paleostriatum is damaged, and within the projection of these nuclei there is a topical organization. While it is probable that the dorsolateral cortical area is not related directly to the thalamus there is evidence that the nucleus superficialis parvocellularis projects to the dorsomedial margin of the hemisphere by way of the septo-mesencephalic tract.

The tectal projection to the brain stem reticular formation in the cat. By G. W. Pearce (*Queen's College, Dundee*) and P. Glees (*University of Oxford*)

In a previous communication we emphasized the importance of the tecto-reticular projection and, using Marchi-stained material, showed that over one-half of the fibres of the crossed tecto-spinal tract end in the brain stem and chiefly in the reticular formation. The present paper is an account of the connexions of the superior corpus quadrigeminum with the reticular formation nuclei as outlined by Olszewski and associates (1949, 1954).

The brain stems of over twenty cats, after survival periods of 4–28 days following lesion of the superior corpus quadrigeminum, have been examined by the methods of Marchi, Glees and Nauta.

In the midbrain, on the same side as the lesion, the nuclei cuneiformis and subcuneiformis receive a large projection from collaterals of tecto-spinal fibres and from direct tecto-reticular fibres. A few fibres reach the nucleus cuneiformis of the other side via the collicular commissure.

Distal to the tecto-spinal decussation the crossed tecto-spinal tract gives fibres chiefly to the nucleus pontis centralis oralis and caudalis and to the nucleus gigantocellularis on the side opposite to the lesion. The ventromedial parts of these nuclei are especially involved and there is some evidence that a few fibres may cross the midline to end in the corresponding nuclei on the same side as the lesion.

The topographical distribution of reticular nuclei receiving fibres from the tectum is very similar to that of areas receiving fibres from the cortex (Rossi & Brodal, 1956) and to those said to influence cerebral activity.

Pathways of protein absorption from cerebrospinal fluid. By David Bowsher. *University of Liverpool*

Homogeneous serum albumen, containing radioactive sulphur, was introduced into the ventricles or subarachnoid spaces (cranial or spinal) of fifteen cats. These animals were killed, mostly 1 or 2 hr. after injection by intra-arterial formalin perfusion, to fix protein *in situ*. Micro-autoradiographs were subsequently prepared from sections through various parts of the neuraxis and meninges.

Protein was found in the arachnoid villi of the superior sagittal sinus, the cerebral and spinal pia mater and the walls of its contained capillaries and veins, and in the subpial cortex; along the sheaths of emergent cranial and spinal nerves; and in the ventricular ependyma, and to a slight extent in the choroid plexus.

Collating these results with earlier physiological studies (Sweet, Bowsher *et al.* 1956), which demonstrated two separate rates of protein absorption from the subarachnoid space, a double pathway of absorption of protein is postulated. The first of these is a lepto-meningo-vascular route, which is believed to be responsible for the rapid component of the absorption curve; the second is a perineuro-lymphatic route, thought to be responsible for the slow component of the curve.

The structure of the parasagittal arachnoid membrane and the Pacchionian bodies. By L. Turner (introduced by G. A. G. Mitchell). *University of Manchester*

There are discrepancies in the descriptions in the current anatomical texts of these structures. Most authors have accepted the description of Weed (1914), who believed that the arachnoid membrane is separated from the pia mater by a considerable interval, the subarachnoid space, and that this space is crossed by trabeculae running, in the main, at right angles to the surface of the brain.

Evidence based on the study of sections from twenty human subjects is presented, to show that in fact the term 'subarachnoid space', applied to the arrangements in this region, is misleading, and that the tissue which lies between the dura mater and the brain is very much more compact than is generally believed to be the case.

Further observations on the anatomy of the lesser curvature of the human stomach with regard to chronic gastric ulceration. By F. S. A. Doran. *Mid-Worcestershire Hospitals*

It is a pathological fact that the majority of chronic gastric ulcers occur on the lesser curvature between 5 and 10 cm. above the pylorus. It is hard to resist the suggestion that this strict localization has an anatomical basis. It has long been suspected that the blood supply to the lesser curvature was weak, but the weakness must lie in the mucosa, because it is known that the ulcer starts as a shallow depression on its surface, and without any pathological condition in the submucosa or muscularis. Here, this weakness is demonstrated to be a tendency for the vascular density of the mucosa along the lesser curvature and in the pars pylorica to be less than that of the greater curvature. This fact might help to explain why the ulceration is confined to the lesser curvature, but it does not explain its restriction to an area between 5 and 10 cm. above the pylorus. Aschoff, however, noted that the mucosa on the lesser curvature was rather taut compared to the rest of the stomach, but he did not measure it. Measurements of the mobility of the gastric mucosa on the muscularis confirm Aschoff's observation, but demonstrate the important feature that it is not uniform, the region of least mobility being between 5 and 10 cm. above the pylorus, exactly coinciding with the observed area in which chronic gastric ulceration usually occurs. It is suggested that this exact correspondence is of definite aetiological importance.

The musculature of the human prostatic urethra. By E. J. Clegg. *Department of Anatomy, University of Liverpool*

The muscles of the prostatic urethra have been studied in transverse sections of six prostate glands, stained by Masson's trichrome method. This part of the urethra can be subdivided into three parts.

(1) Between the internal urinary meatus and the upper part of the urethral crest a thick bundle of smooth muscle fibres, derived from the muscular stroma of the prostate and the longitudinal muscle of the bladder passes transversely behind the urethra. This corresponds to the 'sphincter vesicae internus' of Henle. Anterior and lateral to the urethra are longitudinal fibres derived from the bladder musculature.

(2) Opposite the urethral crest the 'internal sphincter' gives rise to the muscle of the crest, which surrounds the ejaculatory ducts and prostatic utricle as they pass forwards. Near their terminations circular muscle fibres can be seen surrounding these structures, and it is suggested that they may exert a sphincteric action.

(3) Below the entrance of the ducts and utricle into the urethra a bar of muscular tissue occupies the centre of the crest, extending from the 'sphincter vesicae' posteriorly to the submucosa of the urethra anteriorly. At this level the smooth muscle of the sphincter is to some extent infiltrated by striped muscle fibres from the capsule of the prostate, and this part of the muscle probably corresponds to Henle's 'sphincter vesicae externus'.

Accessory bronchiolo-alveolar communications. By Margaret W. Lambert (introduced by C. C. Macklin). *Royal Victoria Infirmary, Newcastle upon Tyne*

In normal lungs from humans of all age-groups as well as in guinea-pigs, rabbits and cats, epithelial lined tubules, hitherto undescribed, can be shown connecting terminal and respiratory bronchioles with certain of the adjacent pulmonary alveoli. Their epithelial lining usually corresponds with that of the bronchioles from which they arise and is continued for some distance over the walls of the alveoli concerned. Their lumina range from about $30\,\mu$ in diameter down to zero and in the case of the terminal bronchioles they may be long and tortuous so that their whole length is seldom included in the one section. Sometimes they are bifid leading to two adjacent alveoli. They are especially inconspicuous in the respiratory bronchioles where they are short and lined by flattened epithelium.

In respiratory bronchioles serial section reconstructions show the tubules to connect as a rule with alveoli arising from their own recurrent branches, but in one terminal bronchiole the tubule has been found to connect with the alveoli of another bronchiolar system. It would appear, therefore, that the tubules, as well as forming short cuts to the recurrent alveoli, may also form links between different lobules.

In human autopsy material the tubules tend to be obscured by peribronchiolar collapse, oedema or cellular debris, and in coal miners they and their associated alveoli often contain clusters of dust cells.

A rare type of congenital cardiac aneurysm.

By E. L. Patterson. *University of Manchester*

Congenital aneurysms of the sinuses of Valsalva and of the interventricular septum are well recognized. Also a number of cases have been reported of an anomalous origin of the left coronary artery from the pulmonary trunk with a resulting apical aneurysm of the left ventricle. The case described is different, being that of a small saccular aneurysm on the diaphragmatic aspect of the left ventricle that ruptured intrapericardially 2 weeks after birth. The aneurysm was found to have a musculo-elastic wall, resembling that of an artery, and was connected with the cavity of the left ventricle by a narrow, endothelially lined sinus passing through apparently normal ventricular myocardium. An embryological explanation for the occurrence of such an anomaly is suggested.

The fine structure of the Pacinian corpuscle. By D. C. Pease and T. A. Quilliam.

University of California, Los Angeles and *University College, London*

An earlier optical study revealed a number of functionally significant features of the nerve ending contained within this sensory end-organ (*J. Physiol.* **129**, 1955), but it proved impossible with the resolution then available to determine whether myelin is present on the nerve terminal, or to describe accurately the arrangement of the encapsulating tissues.

The present electron microscopic studies of transverse sections clearly show that the lamellae of all zones consist of very thin layers of cytoplasm. In the outer zone each lamella, although multicellular, forms an almost perfect circle and each is separated from its neighbours by a fluid-filled space. In the inner core the lamellae form half circles and are arranged in two closely stacked bilaterally symmetrical groups, which are separated from one another by a radial cleft. Slender cytoplasmic processes from cells in an intermediate zone pass centripetally along these clefts and contribute units to either or both of these stacks.

The nerve ending is located axially and so it also separates the two component parts of the central core whose innermost layers lie in intimate relation to its limiting membrane. In outline the ending is usually oval with its longer axis lying coincident with that of the plane of the radial cleft. A peripheral palisade of densely packed mitochondria is a constant feature of the terminal.

Sections taken progressively further proximally demonstrate first of all the acquisition of a Schwann cell sheath and later a myelin coat in addition.

Observations on the structure of digital Pacinian corpuscles (*Corpuscula lamellosa*). By N. Cauna and G. Mannan. *King's College, Newcastle upon Tyne*

Human material from over 100 individuals between birth and 93 years was studied by cytological and nerve-staining methods, together with micro-dissection, wax plate reconstructions and optical polarization techniques.

It was found that digital Pacinian corpuscles change their size, shape and structure from birth to old age. The lamellae of the vascular outer bulb consist of circular and longitudinal collagen fibres embedded in a homogeneous matrix. The cells of the lamellae are modified

fibrocytes which communicate with one another along the surfaces of the lamellae and across the interlamellar spaces. The flattened nuclei of these cells show sex chromatin in female specimens. The lamellae and their cells are derived from the adventitial tissue.

The interlamellar spaces contain loose fibrils of various dimensions arranged in a circular manner. They also contain elongated cells with deeply staining oval or kidney-shaped nuclei and granulated cytoplasm. These cells are frequently connected to the inner or outer surfaces of the lamellae.

The receptor is supplied by one or two thick medullated axons. In prenatal corpuscles, the myelin sheath is lost as the nerve fibre enters the receptor, but later in life the nerve is medullated for some distance inside the corpuscle as a result of retrograde growth of the lamellae around it. The cells of the inner bulb show no distinct boundaries; they are continuous with the neurilemmal sheath, from which they appear to be derived. Fine nerve fibres, sometimes found inside the corpuscle, are derived from the thick axon.

Innervation of the oral mucosa. By A. D. Dixon. *University of Manchester*

The topography of nerve plexuses and the morphology of peripheral nerve terminations in the mucous membrane of the oral cavity have been studied as a basis for further research. Oral tissues from selected regions of a number of mammals, viz. guinea-pig, rabbit, cat, monkey and man, were prepared by several neurohistological procedures including methylene-blue techniques and modifications of the Bielschowsky–Gros silver impregnation method. In many cases the tissues were pretreated with the enzyme hyaluronidase to ensure uniform distribution of the dyestuffs or fixative.

Nerve bundles, as they ascend towards the mucous membrane, give rise to submucous plexuses which vary considerably in their density and fibre arrangement in different parts of the mouth cavity. The plexuses in the cheeks, floor of the mouth and inferior surface of the tongue are composed of small bundles of axons arranged in an open meshwork, while those found in the hard palate and dorsum of the tongue are notable for their greater profusion of axons and extensive ramifications.

Likewise, there are regional differences in the sensory nerve terminations which have their origin in these plexuses, but they are not readily subdivided into distinct morphological groups. They consist of fine non-myelinated terminals which end freely in the epithelium and of many organized endings of varying degrees of complexity, which are located mainly in the summits of the dermal papillae, involving gradations from simple loops to highly complex coiled endings.

The morphological features of nerve plexuses and endings as seen in whole-thickness preparations and frozen sections are described and discussed.

The histochemical appearances of cholinesterase in the submaxillary and sublingual salivary glands of the rat. By R. S. Snell and J. R. Garrett. *King's College, London*

Despite the researches of Babkin (1950) and others, no definite conclusions have been reached with regard to the relative part played by the sympathetic and parasympathetic nerve fibres in the regulation of salivary secretion, or the exact mode of termination of the fibres in the vicinity of the secretory units of the glands. It was considered that a histochemical study of the distribution of cholinesterase in the submaxillary and sublingual salivary glands of the rat might shed light on the problem.

Twenty-four mature rats were used for the present investigation. Frozen sections were processed using a modification of the histochemical technique described by Koelle & Friedenwald (1949).

The results show that true cholinesterase is present in the form of a fine network around the acini of both glands, but is much less evident around the acini of the sublingual gland. The fact that the net unites with the nerve trunks would suggest that it is formed of nerve fibres which contain true cholinesterase. Koelle (1955) found in the cat, rabbit and rhesus

monkey that the concentration of true cholinesterase was higher in cholinergic neurones than in adrenergic and sensory neurones. The present results indicate that the acini of the submaxillary gland of the rat are supplied with a high proportion of cholinergic nerve fibres, whereas those of the sublingual gland are supplied with nerve fibres containing a low concentration of cholinesterase and are thus possibly adrenergic.

The arterial supply of the foetal spinal cord. By J. B. D. Torr.
University of Manchester

The arrangement of the larger arterial vessels around and within the substance of the spinal cord has been investigated in a series of human foetuses whose ages range from 20 weeks to full term. Various injection masses were used, for this aspect of the problem a mixture of latex and indian ink being the most satisfactory. The specimens were then fixed, dissected and in most cases following dehydration were cleared in Tetralin to enable the major vessels penetrating the cord to be seen. In some cases the injection mixture included thorotrast and these specimens were examined radiologically.

It has been found that although the cord arterial circulation presents a basic plan there are some important individual differences and changes which occur with increasing age. In the youngest specimens the vessels follow a comparatively simple, straight course but with increasing age they become much more tortuous. In all specimens the greater contribution to the blood supply by a small number of the radicular arteries is very well marked, as also is the variation in the supply to different regions of the cord.

Demyelinating activity of some organophosphorus compounds and a note on Marchi staining of sciatic nerves. By E. J. Field. *Anatomy Department, University of Bristol*

A number of organophosphorus compounds have been shown in recent years to cause demyelination in chickens and in humans (though not in the rat).

Adult chickens have been given 1 ml./kg. body weight tri-ortho-cresyl phosphate or tri-ortho-ethyl phosphate by mouth, and sacrificed at intervals after the onset of the neurological disturbance which invariably supervenes at 10–14 days (longer with TOEP). Examination has shown:

(*a*) Normal nerve cells in brain and spinal cord.

(*b*) Demyelination in cord especially in the lumbar region, though often more severe in the cervical region of long-standing cases.

(*c*) Variable appearances of demyelination in the sciatic nerves.

The Marchi method used has been a modification of the Swank–Davenport one, kindly suggested to me by Dr Pearce. To test out its constancy, and in order to locate the principal tracts in the chicken cord, the latter has been sectioned and then examined at various levels. Sciatic nerves in chickens and rats have also been stained by this method at 7–21-day intervals after cutting. Whilst the expected pictures were obtained in rats, those in chickens showed many obviously degenerated fibres presenting light brown myelin droplets alongside fully blackened ones.

To test if this could be due to 'inadequate penetration' by the Marchi solution, 20μ frozen sections were stained. Such sections gave quite different pictures from those obtained by block impregnation. No explanation of this can be offered.

Other findings in the chickens were

(1) Vitamin B_1 has no protective effect against TOCP.

(2) TOEP, recently reported in America to be non-toxic, is in fact toxic, though less so than TOCP.

(3) Vitamin E has a distinctly protective effect especially when given by mouth in large doses.

(4) Young birds (up to about 50 days) which appear to be clinically normal when dosed with TOCP may, nevertheless, show degenerative changes in their spinal cords. In explanation it is suggested that demyelination has taken place in tracts not yet used by the bird.

A comparison of staining techniques employed in the investigation of the enteric nerve plexuses. By J. R. Rintoul. *University of Manchester*

Various techniques including methylene blue, silver and specific micro-chemical stains were used and compared. The results obtained by the use of intravital, supravital and combined methylene-blue methods will be shown; and the effects produced by varying pH, and the use of hyaluronidase. Of the silver methods tested the best results in demonstrating ganglion cells and nerve tracts were obtained with modifications of the Bielschowsky technique, and for this purpose the best fixative was found to be 90 % alcohol containing 8 drops of concentrated solution of ammonia/100 ml. Following fixation the pieces were washed for 6 hr, immersed in 12 % neutral formalin and then stained by Gairn's modification of Bielschowsky stain.

The specific micro-chemical stain used was Champy-Coujard's which is reputedly specific for adrenaline-like substances. Photomicrographs of preparations made by the above techniques were demonstrated and the results discussed.

An unusual type of intra-arterial cushion in the arteries of the rat's eye. By D. B. Moffat. *University College, Cardiff*

The main blood supply to the eye of the rat is derived from a branch of the ophthalmic artery. This vessel accompanies the optic nerve and, after entering the back of the eye, gives off the central artery of the retina and then breaks up into the long and short posterior ciliary arteries. The main arterial trunk contains a number of subendothelial 'cushions' which form longitudinal ridges projecting into the lumen, the two main cushions being arranged to form a valve-like structure at the site of bifurcation into central retinal and ciliary arteries.

The structure of the cushions differs from that of those previously described in the dog and cat (Moffat, 1952) in that the main bulk of the 'cushion' is made up of a copious matrix which contains much material of a muco-polysaccharide nature. This substance usually, but not invariably, shows metachromasia when stained with azur A, and is hyaluronidase-labile. The results of other histochemical procedures were described and the possible functions of the 'cushions' discussed.

The presence of a substance rich in protein-bound cystine or cysteine in the neurosecretory system of an insect, *Leucophaea maderae*. By J. C. Sloper. *Charing Cross Hospital Medical School, London*

Comparisons have been drawn between the hypothalamo-hypophysial neurosecretory system of vertebrates and corresponding systems in invertebrates, for example, the pars intercerebralis-corpus cardiacum neurosecretory system of insects (Scharrer, 1953). Such comparisons are supported by the demonstration in both phyla of a deeply staining chrome-alum-haematoxyphil neurosecretory material. In a wide variety of vertebrates personal studies have shown, principally with the aid of the performic acid-Alcian blue technique (Adams & Sloper, 1955), that there is in the exact distribution of this neurosecretory material a substance which is rich in protein-bound cystine (or cysteine). The same histochemical technique selectively demonstrates a similar substance throughout the intercerebralis-cardiacum neurosecretory system of the cockroach, *Leucophaea maderae* (the material for study kindly provided by Prof. Berta Scharrer). On the basis of this observation it is tempting to speculate that a group of neurosecretory proteins exists which

is rich in protein-bound cystine or cysteine and is common to vertebrates and invertebrates alike. This speculation is the more interesting because a number of mammalian posterior pituitary principles have already been shown to be exceptionally rich in cystine (Van Dyke and others, 1942: du Vigneaud *et al.* 1953).

Analysis of experimental suture deviation. By F. G. Girgis and J. J. Pritchard. *Anatomy Department, Queen's University, Belfast*

In different hands experimental damage to the growing skull vault has produced very different effects on the pattern of the cranial sutures at a later date; and this has led to conflicting views about the morphogenesis and functional role of the cranial sutures.

In the present series of experiments on the neonatal rat it has been found that *removal* of the parietal bone without puncturing the dura mater leads to rapid regeneration of the marginal part of the bone, and the suture pattern develops normally. On the other hand, if one parietal bone is *cauterized*, then during subsequent growth the sutures deviate towards the cauterized area. The reason for such deviation has been sought for histologically and by means of vital staining with alizarin injected just before operation. If a small area of the parietal is lightly cauterized near a sutural margin, growth at this margin is arrested, but growth at the sutural margin of the adjoining bone continues unabated into the presumptive territory of the damaged bone, resulting in progressive deviation of the suture, which, however, is histologically of normal structure. These results are in keeping with the view that sutural positions are plastic under experimental conditions, being governed by the rates of growth at the margins of the bones concerned. They also show that whether suture deviation occurs after skull damage or not depends upon the nature of the damage.

Correlation of structure and function in the mesonephros and metanephros of the rabbit. By T. S. Leeson and J. S. Baxter. *University College, Cardiff*

Previous workers, notably Gersh (1937), have investigated the problem of the function of the embryonic kidney in numerous species and have produced some evidence for the function of the meso- and metanephroi before birth.

This question has been studied in the meso- and metanephros of the rabbit by the correlation of ordinary histological staining methods, the periodic-acid-Schiff technique, and both the cobalt and azo-dye methods for alkaline phosphatase on embryos and foetuses of varying ages, 6 mm. c.r. length to birth.

It has been confirmed that the mesonephros in the rabbit is a very large body, present for at least 6 days in a fully developed state. Alkaline phosphatase is present very early in the 'formative' stage before the 'adult' type of distribution occurs, when it is confined to the luminal border of the proximal segment of the uriniferous tubule. During mesonephric development a number of enzymic and histological changes are coincident, and when complete are considered indicative of full development. These same changes occur in the same order in developing metanephric nephrons where probably they are likewise indicative of a functional capacity. The results indicate that the mesonephros is capable of function in the rabbit.

The terminal collecting ducts of the kidney. By F. R. Johnson. *University of Sheffield*

It has long been believed that the terminal collecting ducts or renal ducts of Bellini open freely on the apex of the renal papilla.

Recently, however, doubt has been thrown on the accuracy of this account by claims that (1) the collecting duct does not open freely into the minor calyx, its orifice being occluded by a layer of pelvic epithelium; (2) the terminal collecting duct ends in a cul-de-sac

and its orifice is situated in the wall of the duct at an acute bend which occurs prior to the termination of the duct in the cul-de-sac; and (3) the kidney of the cat contains no ducts of Bellini.

In order to confirm these recent claims a study has been carried out on the terminal collecting ducts in unipapillary and multi-papillary kidneys, including those in the human. The techniques included the study of serial sections of the renal papilla, the injection of the ducts with latex and the micro-dissection of the terminal ducts.

In general the findings confirm the older view that the collecting ducts open freely on the apex of the renal papilla. The arrangement of the collecting ducts is similar in unipapillary and multi-papillary kidneys, and they are also present in the kidney of the cat.

A study of nasal respiratory mucosa. By M. Taylor (introduced by F. Davies). *University of Sheffield*

A study of the normal respiratory mucosa has been carried out in the human, rabbit and rat, as a preliminary to the detailed histological and histochemical study of pathological changes in the mucosa.

Paraffin sections of the mucosa from the nasal septum have been stained by haematoxylin and eosin, Southgate's muci-carmine and by the periodic-acid-Schiff and Gomori's alkaline phosphatase techniques.

In the rabbit and rat, the mucosa is similar in structure. A well-defined layer of basal cubical cells lies beneath the surface columnar cells. The human mucosa is thicker and more irregular in depth than in these animals, and the surface columnar cells are taller, while no layer of basal cubical cells is present; the basement membrane and submucosa are also thicker. In the rat, the mucous glands extend throughout the whole thickness of the submucosa, while in the human and rabbit these glands are found only in the deeper half.

With the periodic-acid-Schiff technique the cytoplasm of all the cells of the epithelium, including the goblet cells, gives a positive reaction in all cases. In addition, in the human, the cytoplasm of the cells of some of the larger ducts, the basement membrane of the epithelium and the mucous glands show a positive reaction. In the rabbit the mucous glands and also the elastic lamina of the arterioles show a positive reaction, whereas in the rat most mucous glands are negative, but the basement membrane round the glands shows a positive reaction. These appearances do not differ after incubating with saliva, indicating that glycogen is not a normal constituent of the mucosa. Southgate's muci-carmine gives similar appearances.

The cytoplasm of the epithelium, the goblet cells and the endothelium of the blood vessels show a positive reaction for alkaline phosphatase; the reaction is uniformly weak at short incubation periods but is more positive at longer incubation periods (3, 6 and 24 hr.).

Alkaline phosphatase reaction of the myoepithelial cells in the rat submaxillary gland. By C. R. Leeson and F. Jacoby. *University College, Cardiff*

A study has been made of the distribution of alkaline phosphatase in the rat submaxillary gland both by the method of Gomori and by a modified azo-dye method. With incubation times of 2 hr. or more in the Gomori method there was a heavy blackening around and at the base of the acini which appeared to confirm the observations of earlier workers. That this might be the result of diffusion seemed possible and with shorter incubation times a regular pattern of alkaline phosphatase-positive material in the gland has been observed. This pattern points to an almost specific localization of alkaline phosphatase in the myoepithelial cells. These cells are present around the short intercalated ducts as well as around the acini.

Similar studies on the submaxillary glands of the guinea-pig and the rabbit have failed so far to demonstrate myoepithelial cells by this method.

A study of the finer structure of the myoepithelial cells in the rat submaxillary gland, with the aid of the electron microscope, is in progress. Preliminary findings are described.

Craniometry: a suggested new method. By D. I. G. Bunn and P. Turner (introduced by E. W. Walls). *Middlesex Hospital Medical School, London*

In physical anthropology an index is a convenient way of expressing one measurement as a percentage of another, each of many recognized indices being thus derived from four points. Although in combination a series of indices can give some overall idea of form, the precise spatial relationships of the various sets of landmarks between which measurements are made remain obscure.

A system, based on Cartesian co-ordinates, has been devised for analysing skull-shape and size—including the spatial relationships of selected landmarks. From chosen points on the skull, measurements are made to the Frankfurt plane, to the sagittal plane and to the 'third' plane which passes through the external auditory meati at right angles to the first two.

The following indices have been employed: x/d, y/d, z/d (angular indices) and d (linear index), where x, y and z are the distances of a point on the skull from the Frankfurt, sagittal and 'third' planes, respectively, and d is the distance from a reference point called the Homologous Reference Point (H.R.P.), which lies at the intersection of the three planes: $d = \sqrt{(x^2 + y^2 + z^2)}$. The general index d/s, where $s = \Sigma d/n$ can also be used.

The H.R.P. is not a particular point on the skull and its position does not need to be known, but for certain investigations, e.g. growth studies, it could be adjusted to coincide with a fixed point.

The apparatus and method of skull orientation are as described by Bunn & Turner (*J. Anat.* **90**, 1956). Distances from the Frankfurt plane are recorded as positive if above and negative if below; from the 'third' plane as positive if anterior and negative if posterior; from the sagittal plane always positive.

Sample indices obtained by the method are shown, and the application of the method to other bones discussed.

Adherences between penis and sheath in castrated oxen. By R. R. Ashdown (introduced by C. W. Ottaway). *University of Bristol*

Bull calves are usually castrated while separation between penis and sheath is still proceeding (i.e. before 10 months). Castration inhibits this separation and the penises of adult castrates show differing degrees of fusion.

The penises of 207 castrates were examined and classified according to the degree of fusion between the penis and its sheath: 27 % were completely separate; 29 % were fused over the 'collum glandis' only; 41 % showed considerable separation over the apex (especially the 'galea glandis'); 3 % were separate only in the region of the urethral process. Arrangement of material in order of increasing separation revealed progressive freeing of: urethral process, 'galea glandis', the rest of the apex, 'collum glandis', and finally a small dorsal area at the base of the free end. It seems probable that this sequence is that of normal separation.

The ages of 161 of these animals ranged between $1\frac{1}{2}$ and $3\frac{1}{2}$ years. There was a slight trend relating age to degree of separation ($P < 0{\cdot}10$, $> 0{\cdot}05$); in a large proportion of older animals separation was complete. It has been shown that castration of puberal and adult mice is followed by refusion of penis to sheath (Raynaud & Chaulin-Servinière, *Bull. Soc. zool. Fr.* **67**, 1942). This survey indicates that refusion does not follow castration in the ox; on the contrary, the relationship between age and separation suggests that post-castrational inhibition of separation may not be complete.

The effect of alloxan and partial pancreatectomy on pancreatic grafts.

By Rex E. Coupland. *University of Leeds*

Pieces of pancreas obtained from foetal (over 19 mm.) and day-old rats were implanted into the anterior chamber of the mother's eye. The graft became implanted on the iris. Homograft reactions did not occur. Acinar tissue disappeared while Islets of Langerhans persisted, together with a variable amount of duct epithelium, lymphatic and fatty connective tissue. In some cases a small amount of developing spleen was inadvertently included in the graft, and in three cases a typical small 'spleen' 2–3+ mm. in diameter developed.

The grafted Islets of Langerhans persisted for many months and contained cords of cells, which could be differentiated as α and β types by azo-carmine and aldehyde-fuchsin stains.

Alloxan 40 mg./kg. body weight was injected intravenously into four animals 2–3 months after the implantation of the graft. The β cells of both normal pancreas and graft underwent degeneration.

In six animals a partial pancreatectomy was performed. This resulted in a proliferation of the grafted islets—as judged both by the presence of mitotic figures and by an increase in number of individual islets.

The relationship between the antigens of cartilage and skin in the rabbit.

By M. B. L. Craigmyle. *University College, Cardiff*

It has been suggested (Loeb, 1930) that cartilage homografts survive because they do not elicit an antibody response in the host. In a previous paper read before the Society it was shown that changes in the regional lymph node induced by a cartilage homograft appear to invalidate this hypothesis (Craigmyle, 1955). It has been shown that, in dogs, a skin homograft will sensitize the animal to a subsequent kidney or spleen homograft taken from the same donor, and vice versa (Dempster, 1953). Thus, the reaction to a homograft is individual-specific, not tissue-specific.

The fate of a skin homograft has been studied in twenty rabbits, and compared with that of a skin homograft in a further twenty rabbits, each of which had had a prior implantation of cartilage taken from the same animal which supplied the skin. Evidence of accelerated breakdown in a large proportion of the skin homografts in the latter group indicates the ability of cartilage homografts to sensitize a rabbit to a subsequent skin homograft from the same donor. The significance of the findings is discussed.

The fate of autografts of ileum inserted into the bladder wall in the rabbit.

By J. Joseph and G. A. Thomas. *Guy's Hospital Medical School, London*

There have been several reports of the use of the ileum to replace the diseased bladder wall in man, but the detailed histology following these procedures has not apparently been studied. In the present investigation two types of experiment were carried out. In one series of rabbits a completely separated piece of ileum was sewn into the posterior wall of the bladder (referred to as a 'patch graft'), and in the other a loop of ileum was isolated, its blood supply retained and the loop sewn on to the bladder after the upper part of its wall had been removed ('pedicle graft').

In the series with a patch graft the bladders were removed after 7, 11, 21, 35 and 49 days and examined histologically. The patch graft showed the following features. The ileal mucous membrane disappeared by 21 days and was replaced by transitional epithelium by 35 days. The rest of the ileal wall was replaced by fibrous tissue during the same period. In the second series the pedicle graft and bladder were removed after 20, 30, 40 and 50 days. Histological examination showed in all cases that the ileum was integrated into the bladder wall and had retained its complete structure. The mucous membrane was intact and all the layers of its wall were apparently normal. Histochemical examination is being carried out.

FEBRUARY 1957

An ordinary Meeting of the Society for the Session 1956–7 was held on Friday, 22 February 1957 in the Department of Anatomy, St Thomas's Hospital Medical School, London, S.E. 1. The President (Prof. R. D. LOCKHART) was in the Chair for the morning and afternoon sessions and Prof. R. M. BOWDEN for the evening session.

The following are the authors' abstracts of the papers presented:

The absorption of oleic acid from the small intestine of the rat. By W. HEWITT. *St Thomas's Hospital Medical School, London*

During the absorption of neutral fat, phospholipid is formed in the cells of the intestinal epithelium. It has been suggested, by some authors, that this substance acts as an intermediary in the re-formation of neutral fat from the fatty acid derived by the hydrolysis of the ingested fat. Using histochemical techniques the intestinal mucosa of the rat has been studied $2\frac{3}{4}$ hr after feeding with triolein and the results of these experiments have been previously reported. These results give grounds for believing that a possible relationship may exist between the amounts of neutral fat and phospholipid which appear in the cells, but it is possible that the neutral fat may have been derived from the ingested fat which had not been hydrolysed. Accordingly, the experiments have been repeated, but with the exceptions that oleic acid was substituted for the triolein and the intestinal cells were also examined 1 hr. after feeding.

The results of these experiments are identical with those where triolein has been administered and, as before, neutral fat, fatty acid and phospholipid could be detected inside the cells. After feeding, however, fatty acid is most abundant 1 hr. later, whereas neutral fat and phospholipid predominate $2\frac{3}{4}$ hr. later. The relationship between the amounts of neutral fat and phospholipid present still prevails.

The response of the mammary gland of the weanling male mouse to the injection of hormones concerned in mammogenesis. By J. I. H. HADFIELD (introduced by D. V. DAVIES). *St Thomas's Hospital Medical School, London*

Experimental work carried out over the last 10 years suggests that the anterior pituitary trophic hormone responsible, with oestrogen and progesterone, for the normal growth and development of the mammary gland is either prolactin or another protein hormone that comes down with prolactin during its preparation from pituitary extracts.

A routine method for staining the mammary glands and analysing the results quantitatively has been established.

Investigations are being carried out into certain aspects of this problem using the mammary glands of intact weanling male mice injected with a series of hormones and endocrine extracts twice daily for a period of 5 days. Growth responses have been estimated by counting the average number of terminal clubs per mammary gland in each batch of treated animals. The average number of terminal clubs per gland in normal weanlings of the strain used is 1·2.

The oestrogen steroids, using oestrone at an optimal dose level, produced a growth response whose numerical assessment presented no difficulty. This steroid over a period of 5 days produced an easily recognizable growth of the duct system with no glandular differentiation.

A crude extract of placenta gave a similar result.

Prolactin, on the other hand, over the same period induced considerable formation of acini in the clubs, in addition to the formation of a primitive duct system.

Ossification of the patella. By J. McKenzie and E. Naylor. *University of Aberdeen*

Five X-rays of the patella of a healthy boy taken at yearly intervals showed multiple centres of ossification ultimately coalescing to form a normal bone on each side.

Although orthodox British textbooks of anatomy maintain that the patella is ossified from a single centre, Hellmer (*Acta Radiol.*, Supplement XXVII, 1935) clearly demonstrated in his series of over 400 cases that the patella always ossifies from several centres and in radiological practice multiple centres of ossification are regarded as a normal occurrence in this bone.

Observations on the growth of the cranial base. By E. H. R. Ford. *University of Cambridge*

Measurements on a collection of immature human skulls confirm recent observations that the segment of the cranial base from the pituitary fossa to the foramen caecum does not grow after the age of 7 years. Subsequent increase in length of the anterior part of the cranial base is due solely to increase in thickness of the frontal bone.

The cribriform plate of the ethmoid hardly increases in length after the age of 2 years, and is the same width at birth as in the adult. Bony union between the perpendicular plate and the lateral masses of the ethmoid is established by the end of the first year.

The relation of the nasion to the plane of the sphenoidal jugum and the cribriform plate varies considerably between individual skulls. Most of the relative upward movement of the nasion which occurs during growth takes place during adolescence.

Observations on premature fusion of the sagittal suture. By B. H. Dawson and D. A. N. Hoyte. *University of Manchester*

The clinical and radiological features of a series of fourteen children with sagittal synostosis have been examined.

When the sagittal suture is prematurely obliterated, lateral growth of the cranium is restricted so that the skull becomes abnormally narrow. Compensatory overgrowth then occurs in the long axis of the skull. The mechanism of production of abnormal cranial contours in these patients is described and illustrated.

Examination of radiographs taken at different ages shows that the process of abnormal sagittal suture obliteration follows a fairly constant pattern. An excessive accumulation of bone on the endocranial aspect of the sutural margin is observed and the normal sutural gap is narrowed and then obliterated. At the same time or later the gap is bridged over by a growth of bone. A denser ridge of bone now occupies the position of the normal suture.

These findings are examined in the light of recent work on the anatomy of the cranial sutural regions. It is suggested that this abnormality can be explained by a failure of bone resorption on the cerebral aspect at the margins of the sutural gap.

Further observations on the mandibular meniscus in rabbits. By R. Sprinz. *University of Sheffield*

Six-week-old animals were used. The meniscus was subjected to the following operations: (*a*) partial incision extending from the periphery to about the centre, but not extending through the whole thickness of the disc; (*b*) simple division of the disc of similar extent, but passing through the whole thickness; (*c*) excision of small segments of the disc. The animals were sacrificed after 8 weeks and the discs examined histologically. Partial incision (*a*) of the disc was followed by fibrous tissue repair without any change in the mandibular condyles. Simple division (*b*) of the disc was also repaired by fibrous tissue in one, but not repaired in another case. In both these cases as in those involving partial excision (*c*), the

condyles showed enlargements similar to those previously described (Sprinz, *J. Anat.* 88, 1954) that follow complete meniscectomy. No repair of the disc ensued after partial excision in the present series.

Control operations were performed in which the superior and inferior compartments of the joint were separately and conjointly opened, the synovial fluid being released. The results of these operations on the mandibular condyle were different from those described above. Here, part of the articular surface of the condyle showed slight indentations. The shape of the condyle remained essentially unaltered.

It is, therefore, suggested that the mandibular meniscus in the rabbit has a very limited repair potential on the grounds that no regeneration occurs after the removal of very small segments of the disc. When no regeneration of the disc occurs, the condylar enlargement is not due to the operative procedure.

The annular ligament of the superior radio-ulnar joint.

By B. F. Martin. *University of Sheffield*

A study of the annular ligament by dissection of thirty specimens established that this ligament is much more complex than has previously been indicated. It was found that several ligamentous bands contribute to the formation of the annular ligament posteriorly, and that they arise from the ulna somewhat behind the radial notch. The upper portion of the annular ligament, i.e. the portion surrounding the head of the radius, was formed from a single ligamentous band in only about one-half of the specimens; in the remainder, the ligament was in two or more parts, and not infrequently was joined from above by a separate ligament arising opposite the middle of the trochlear notch. Below the level of the radial notch, the ligamentous arrangement was somewhat variable, but in all cases a strong ligament originated from the upper part of the supinator crest, often from a well marked tubercle; usually, but not always, the bulk of its fibres crossed the annular ligament to blend with the lateral ligament of the elbow joint, the remainder continuing forwards into the lower part of the annular ligament, i.e. the part applied to the neck of the radius. However, the main contribution to this lower part of the annular ligament was found to come from a separate band, situated deep to the former ligament.

Anteriorly, the upper part of the annular ligament formed a single band attached near the radial notch, but an accessory ligament of variable prominence was constantly present, arising a few mm. medial to the lower part of the radial notch, its fibres passing laterally into the lower part of the annular ligament.

Some incidental observations made on the oblique cord suggested that it represents a degenerate part of the deep head of the supinator muscle.

The structure of the mesonephros of the rabbit by electron microscopy.

By T. S. Leeson. *University College, Cardiff*

The 17-day mesonephros of the rabbit has been studied after fixation in 1 % buffered osmium tetroxide. Thin sections of the embedded material were cut on a heat-advance ultramicrotome. The embedding medium used was 5 % methyl in 95 % *n*-butyl methacrylate or 'Araldite', an epoxy-resin. Sections were mounted on carbon or colloidin films and examined with a Metropolitan-Vickers E.M. 3 microscope.

The similarity between the mesonephric glomerulus and that of the adult metanephros is most striking. In the mesonephros, the capillary loops are lined by a thin, attenuated layer of endothelial cytoplasm around which is a basement membrane which, at the resolution obtained, appears structureless, as does that of the metanephros. Firmly attached to the basement membrane are numerous interlacing pedicels of the visceral epithelial cells or podocytes. Trabeculae of these epithelial cells are marked. The proximal segment of the tubule shows a brush-border, and one feature of particular interest is the presence of large vacuoles which may be identical with the fluorescent granules as described

by Sjöstrand. The luminal bodies of cells of the distal segment of the tubule show the presence of sparse, small microvilli. These findings are discussed with reference to the function of the mesonephros as a urinary organ.

Salivatory pathways and the olivo-cochlear bundle of Rasmussen.
By C. C. D. Shute. *University of Cambridge*

G. L. Rasmussen (1946) presented experimental evidence that nerve bundles, which had been previously known as the superior olivary peduncle, and the medial or crossed root of the vestibular nerve, in fact formed part of a crossed tract arising from retro-olivary cells and reaching the spiral ganglion of the cochlea via Oort's anastomosis. He believed that these bundles were not related to salivation, and cast some doubt on the validity of previous work on the salivatory centres and pathways.

The present study has been made on silver-impregnated rat, rabbit, pig, deer, cat and human embryos. In non-human material the viscero-motor fibres of the nervus intermedius often stain differentially. They leave the brain stem not with the sensory portion of the nerve but in company with the vestibular rootlets of VIII, reaching the facial trunk probably by way of the original connexion between the vestibular and geniculate ganglia. Their central course appears to accord with the experimental findings of Kohnstamm (1902) and Kaida (1929). It is concluded that the medial root of the vestibular nerve is salivatory at least in part, and that the decussation of the facial colliculi is not derived entirely from the olivary peduncle, but also receives the axons of cells located in the region of the superior salivatory nucleus. Some of the decussating fibres enter the facial genua, and those situated most anteriorly appear to form a crossed root of motor V, as described by Obersteiner (1888).

It is suggested that further experimental work is needed to exclude the possibility that Rasmussen's bundle is formed by aberrant salivatory fibres.

The lymphocyte in guinea-pig bone marrow. By J. M. Yoffey and P. F. Harris. *University of Bristol*

Quantitative estimates of thoracic duct lymphocyte production in the guinea-pig indicated a Daily Replacement Factor of about 3·0 (Reinhardt & Yoffey, *J. Physiol.*, in the press), and further work has indicated that the bone marrow contains about eight times as many lymphocytes as daily enter the blood via the thoracic duct. The lymphocytes in normal marrow are not organized in nodules, but are scattered diffusely throughout the marrow parenchyma. Frequently, there is an accumulation of lymphocytes in the marrow sinusoids, and these are believed to be myelopetal, not myelofugal, though the mechanism by which such an accumulation could occur cannot be explained.

Lymphocytes in guinea-pig marrow are virtually all small lymphocytes, with occasional transitional forms which appear to be intermediate between the small lymphocyte and the blast cell. These transitional lymphocytes are especially numerous in marrow recovery after irradiation, though they can also be seen in appreciable numbers in normal marrow. The marrow lymphocyte has a small amount of cytoplasm at one pole, with an extremely high N:C (nucleus to cytoplasm) ratio. In the transitional cells the nucleus becomes leptochromatic, the cell enlarges considerably, and only then as a rule does the cytoplasm begin to increase and become basophilic. Mitoses of blast cells are not frequent, and the cells are few in number. It is probable, therefore, that the blast cell stage is of short duration.

Measurements of the volume of bone marrow in guinea-pigs.
By George Hudson. *University of Bristol*

Knowledge of the size of the bone marrow, considered as an organ, is essential for quantitative study of its haemopoietic activity.

The method used was that of measuring the volume of marrow spaces in the macerated

skeleton by means of density bottles. After the volume of its bone substance had been measured, the interior of the skeleton was filled with agar and its total volume estimated. The difference between these two measurements represented the volume of agar taken up and hence, after correction for agar uptake of marrow-free bone (estimated for each skeleton), the volume of marrow spaces. Validation experiments indicated that this was a reliable estimate of marrow volume.

In 25 animals of 350–450 g. body weight, the mean marrow volume was 7·014 ml. ($s = 0{\cdot}358$).

In 120 animals of 85–1250 g. weight, there was a linear relationship between marrow volume and logarithm of body weight ($b = 7{\cdot}885$. $s_b = 0{\cdot}171$); also between marrow volume and logarithm of macerated skeleton weight. Approximately half of the marrow was found in the limb bones, a fifth in the skull, and the rest in the trunk.

In 12 animals of 350–450 g. weight, the mean red marrow volume was 6·249 ml. ($s = 0{\cdot}446$), approximately 89 % of total marrow and a third of liver weight. The limb bones of one side were used to observe the limits of the fatty marrow (it was confined to the distal part of limbs and coccyx), the other side being used for marrow volume estimation.

The structure and regeneration of epithelium in the oesophagus of the cat.

By R. M. H. McMinn and F. R. Johnson. *University of Sheffield*

Histological and histochemical studies of the epithelium of the lower end of the oesophagus showed that the epithelium can be divided into three zones: a superficial, with the cells squamous in type and with flattened pyknotic nuclei; an intermediate zone of large polyhedral cells with round vesicular nuclei; and a basal zone of small closely packed polyhedral cells.

Regeneration was studied following the production of artificial ulcers by removing a small area of mucosa through an incision in the abdominal portion of the oesophagus. The regenerative process was characterized by a migration of cells over the floor of the ulcer in the form of a band of cells several layers thick, and by an increased rate of mitosis in the surrounding epithelium. During this process the basal cells became enlarged and the more superficial cells underwent degeneration. Later the normal stratification was restored. In the undisturbed mucosa, alkaline phosphatase was shown by the Gomori cobalt-sulphide technique and by the azo-dye method to be localized in the superficial and basal zones of the epithelium. These reactions were always negative in the regenerating epithelium, and reappeared first in the superficial layers following complete epithelialization. The superficial zone in normal epithelium gave a positive reaction with the PAS technique following incubation with diastase; this reaction disappeared at the ulcer site during the period of regeneration. Although glycogen was never detected in the normal epithelium, large quantities were seen in the cells of the intermediate zone at the margins of the ulcers, and in the middle layers of the spreading cells. As epithelialization became complete, glycogen disappeared. Staining with toluidine blue and with methyl-green pyronin confirmed the stratified pattern of the normal epithelium and demonstrated that this disappeared during rapid growth.

These findings were compared with those occurring during regeneration in other epithelia in this animal.

Experiments on the interrelationship of macrophages and fibroblasts.

By F. Jacoby. *University College, Cardiff*

The problem of the interrelationship, i.e. mutual transformation, of macrophages and fibroblasts is still subject to controversy. An account of various experiments is given, designed to throw further light on this problem. They are all tissue culture experiments employing chick cells. They deal, in turn, with long-term observations on pure populations of blood macrophages; with continuous serial photographic records of mixed cell populations;

with the part played by mitotic activity of the macrophages in such mixed populations; and with cell elimination tactics applied to mixed populations and subsequent tests on the surviving cell type. From all these different experiments it appears that, under these conditions, the two cell types are fixed, and no evidence of mutual transformation has been obtained.

The extrinsic innervation of mammalian hair follicles.

By A. D. Dixon. *University of Manchester*

An examination of skin impregnated with silver after removal from the upper lip of the rat, rabbit, cat and monkey reveals two distinct ways by which nerve fibre bundles approach the hair follicles.

In relation to vibrissal hairs thick nerve bundles, composed of a large number of stem fibres, ascend towards the apex of the follicle, pass through the outer fibrous coat and spread out fanwise to terminate in the outer root sheath.

In contrast, smaller, less specialized hair follicles are innervated by stem fibres which descend from the cutaneous nerve plexus to reach the neck of the follicle immediately below the secretory portion of the sebaceous glands. The fibres then terminate as fine fibrils which either encircle the follicle or turn sharply upwards towards the skin surface on the outer root sheath.

These differences, which are common to all the species studied, may have an embryological explanation. As the cutaneous nerve plexus is being elaborated primary, or vibrissal, hair follicles have reached an advanced stage of differentiation, and thus receive a direct innervation from the main fibre bundles as they extend towards the skin surface. The secondary ectodermal downgrowths, which give rise to the smaller, less specialized hair follicles, differentiate at a later stage and as they do so invaginate the cutaneous nerve plexus from which they are innervated.

The embryological development of the anterior spinal artery in man.

By J. B. D. Torr. *University of Manchester*

The development of the anterior spinal artery has been studied in a series of human, sheep and pig embryos. The human embryos ranged in size from 5–60 mm. In the earlier specimens the findings of previous workers have been confirmed, namely, that primitive paired anterior spinal arteries are developed from bilateral capillary nets which cover the ventrolateral aspect of the developing spinal cord but leave the centre devoid of vessels. Later some communications are established across this central area and the medial margins of the net enlarge, forming two longitudinal vessels.

The change from paired anterior spinal arteries to a single vessel has been seen to take place in the range from 15 to 22 mm., the single vessel being produced throughout most of its length by the movement medially of the two vessels and finally their fusion together and not, as has been previously suggested, by the obliteration of different parts of the two vessels with the final selection of one path.

Observations on the autonomic interstitial cells in the gut wall.

By J. R. Rintoul. *University of Manchester*

Methylene blue, silver and Champy-Coujard staining techniques have been employed to study the nature of the autonomic interstitial cells in the gut wall in the rat, rabbit, guinea-pig and monkey. Preparations are shown which support the view that the autonomic interstitial cells are nervous elements.

(1) Their morphology is similar to that of nerve cells.

(2) The autonomic interstitial cells (A.I.C.) have connexions with

  (*a*) type II neurones of Dogiel,

  (*b*) the smooth muscle cells of the gut wall.

(3) The A.I.C. and their processes can be stained by a micro-chemical technique (Champy-Coujard) which demonstrates specifically the presence of adrenaline-like substances.

These findings lend support to the theory that the A.I.C. form a terminal nervous network.

The effect of nerve section on the histochemical appearances of cholinesterase in a mixed peripheral nerve. By R. S. Snell. *King's College, London*

Twenty-four mature guinea-pigs were used for the investigation. In twelve of the animals the right sciatic nerve was cut in mid-thigh and a portion 1 cm. in length was removed. In the remaining twelve animals the right sciatic nerve was also cut, but the two ends were immediately approximated by means of a single silk suture. Animals from each group were killed at intervals over a period of 6 weeks following the operation. The cut sciatic nerve was removed from each animal and at the same time the normal nerve was removed from the other leg to serve as a control. Frozen sections of the nerves were processed using a modification of the histochemical technique described by Koelle & Friedenwald (1949).

The results show that in the normal sciatic nerve cholinesterase is present in moderate amounts in the axons and neurilemmal sheaths. In the nerves which had been cut the concentration of cholinesterase in the proximal portion remained normal, while in the distal portion the cholinesterase became reduced in amount as degeneration proceeded. When regeneration commenced the nerve fibrils growing out of the proximal stump were found to contain a very high concentration of cholinesterase, much greater than that seen in the normal nerve: and similar appearances were noted in the nerve fibrils growing into the peripheral neurilemmal tubes in the nerves which had been cut and sutured. The significance of these findings is discussed.

Cholinesterase activity in the dog heart wall. By R. L. Holmes. *University of Leeds*

Pieces of dog atrial wall trimmed to leave the whole endocardium and part of the adjacent myocardial layer were treated according to the Koelle method for the demonstration of cholinesterase activity. When acetylthiocholine was used as a substrate, activity was observed in the following sites:

(*a*) Distributed diffusely throughout the muscle.

(*b*) In a few ill-defined but localized areas, possibly at the terminations of thick myelinated nerve fibres (receptor endings).

(*c*) In a pattern corresponding closely to that of fibres of the subendocardial nervous network (terminal reticulum).

There was a general lack of precipitate in thick myelinated nerve fibres and only doubtful demonstration of their endings (*b*). This may be due to failure of the substrate to penetrate the lipoid barrier of intact fibres. The positive reaction in the network appeared to be localized to the processes of cells (autonomic interstitial cells), whose nuclei remained clear of precipitate. Material incubated with a butyrylthiocholine substrate, or a substrate containing eserine, showed none of the positive results described.

These findings indicate that the terminal reticulum contains acetylcholinesterase, and reinforce the view that it is a nervous structure and not connective tissue.

The histochemical localization of specific cholinesterase in the lymphatic tissue of the cat. By N. D'Agostini and B. Rossatti. *University of Ferrara*

The histochemical localization of cholinesterase in the lymphatic tissue of the cat has been studied in young and adult animals by means of the thiocholine method of Koelle & Friedenwald (1949), modified by Gerebtzoff (1953). The findings in lymph nodes of different regions, in the spleen and in the palatine tonsil are reported. The chief site of specific cholinesterase activity is found in the germinal centres of the lymphatic nodules

of all these organs. Such enzyme activity appears to be concentrated in the walls of the small blood vessels supplying the germinal centre. In sections of 30–50 μ, the positiveness of the histochemical reaction is demonstrated by an irregular black network in the central portion of the nodule, corresponding to the vascular patterns of the nodule itself. Endothelial cells, reticular and collagenous fibres of the wall of capillaries in the germinal centre show high specific cholinesterase activity. Arterioles situated in proximity to lymphatic nodules exhibit moderate enzymatic activity.

In the parenchymal cells, the cholinesterase activity extends to the layer of lymphocytes in contact with the wall of the blood vessels of the germinal centres of lymphatic nodules. Such a positive reaction, limited to cells surrounding the vascular wall, is probably due to enzymatic diffusion. The germinal centres of lymphatic nodules can exhibit different degrees of enzymatic activity.

Cartilage induction by devitalized muscle in the rabbit. By J. B. Bridges and J. J. Pritchard. *Queen's University, Belfast*

In a previous communication it was reported that alcohol-killed skeletal muscle regularly induced the formation of cartilage when implanted under the kidney capsule of the rabbit. Further investigation has shown that similar cartilage induction follows implantation of parts of the alimentary canal and bladder wall, and the fact that results are unaffected by the removal of epithelium before implantation strongly suggests that the inductor is present in plain muscle. Equally good results have also been obtained with cardiac muscle.

This demonstration of the widespread distribution of cartilage-inducing powers in all kinds of rabbit muscle affords an explanation for the reports of Heinen, Dabbs & Mason (*J. Bone Jt. Surg.* **34** *a*, 1949), and others, that cartilage and bone may develop heterotopically after the injection of alcohol into the limb muscles of rabbits. It is believed that in such cases alcohol acts as a non-specific necrotizing agent, and the specific inductor is liberated from the devitalized muscle to act on the young inflammatory connective tissue which appears as a result of damage to the muscle.

Stereo colour transparencies as an aid to embryology. By W. R. M. Morton. *Queen's University, Belfast*

The visualization, and presentation to others, of organogenesis in small embryos is greatly assisted by stereoscopic colour transparencies in which the position and relations of the organs are at once apparent. The comparison of dissected embryos of varying ages and sizes results in a quicker and better understanding of growth changes than can usually be obtained from a brief examination of serial sections of similar embryos. A simple and inexpensive method of producing satisfactory stereo-photographs, using 35 mm. colour reversal film, is briefly described, and examples of such photographs in hand-viewers are demonstrated.

APRIL 1957

An ordinary Meeting of the Society for the Session 1956–7 was held on Friday, 26 April 1957, in the Department of Anatomy, Royal Free Hospital School of Medicine, London, W.C. 1. The Chair was taken by Prof. R. E. M. Bowden and the President (Prof. R. D. Lockhart) successively.

The following are the authors' abstracts of the papers read:

Nerve endings in human laryngeal muscles. By M. F. Lucas Keene. *Royal Free Hospital School of Medicine, London*

The material examined includes serial sections of two left cricothyroid muscles and one left crico-arytenoideus posterior, all cut transversely. One of the above blocks also exhibits parts of transverse and oblique arytenoideus, thyroarytenoideus and the left crico-arytenoideus lateralis.

Further, a right crico-arytenoideus posterior and a right cricothyroid muscle are cut in serial section in the long axis of their fibres.

Alternate slides of one block have been stained by Romanes's Silver Method and haematoxylin and Biebrich scarlet. All the other sections have been stained by Romanes's Silver Method. All sections have been cut at 15μ.

A preliminary survey of the above material comprising nearly 1000 slides establishes the fact that the muscles are very richly supplied with nerves, and that numerous muscle spindles (as well as other nerve endings) are present in all the specimens.

The scope of future work includes observations on the pattern of distribution of the muscle spindles in the various muscles and the sources of the nerve fibres of the spindles, especially regarding their relations to motor nerves supplying the fibres.

Histological development of the testis in the domestic cat. By M. G. Scott (*Royal Veterinary College*) and P. P. Scott (*Royal Free Hospital School of Medicine, London*)

The characteristic features of the development of the testis, which are described and illustrated, have been studied in material obtained from fifty-six normal cats, bred and reared in the R.F.H.S.M. Colony, ranging from 55 days foetal age to 4 years old. Ten adult cats of unknown age have also been investigated. The material has been fixed in Allen's modification of Bouin's fluid and in Gendre's fixative. Sections have been stained by routine methods in order to study general structure and the distribution of nucleic acids, glycogen and lipids. Five phases of development are apparent: prenatal; postnatal (birth to 7th week); postweaning (7th to 12th week); prepuberty (12th to 20th week); and the phase covering the onset of spermatogenesis (20th to 36th week).

In healthy cats, whose weights fall within the normal range determined for the colony, the early stages are related to body weight, but during the onset of spermatogenesis, the cytological picture presented is more closely related to the weight of the testis than to body weight.

Observations on premature fusion of the coronal suture. By B. H. Dawson and D. A. N. Hoyte. *University of Manchester*

Children with premature obliteration of the coronal suture show changes both in the vault and in the base of the skull. The nature of these deformities has been studied in the radiographs of five affected children. The anatomical features of these compensatory changes are described and illustrated.

As a result of the diminished capacity of the anterior cranial fossa, there follows a compensatory expansion of the middle cranial fossa. The middle fossa bulges forwards, laterally

and downwards, and the sphenoid bone is rotated. The spheno-occipital synchondrosis is widened. The orbit also is distorted, assumes a more oval shape and is more obliquely placed.

These abnormal features may throw light upon some processes of normal skull growth.

The interpretation of the results of alizarin staining of bone in growing animals. By D. A. N. Hoyte. *University of Manchester*

A 2% solution of sodium sulphalizarate has been given by intraperitoneal injection to growing rodents, in a single dose varying from 50–100 mg./kg., and the animals sacrificed at intervals thereafter.

The red dye is found in a distinct layer of bone. In the long bones the fate of the red metaphysial cone and of the subperiosteal alizarin line can be followed in serial transverse sections. Even if both lines have been resorbed, the changes which occur in the bone between them can be traced by noting the presence of stained Haversian systems and of reversal lines. Resorption of bone is not always shown by the presence of Howship's lacunae; the surface may be quite flat.

In the bones of the skull vault red lines are seen marking the original inner and outer tables. Red trabeculae or occasional Haversian systems 'label' the area between the tables, and their subsequent development or removal may be traced. The inner and outer tables may be joined together at or near the sutural edge by a 'closing' or 'joining' trabecula. When seen, this can be taken as a fixed point. In its absence, no conclusion can be reached as to the previous edge of the bone; no macroscopic view of the remaining red edge can be taken as a fixed margin; no measurements from such a margin can give an accurate representation of sutural incremental growth.

The so-called osteogenic fibres of Ranvier's ossification groove and their relation to the fibres of perichondrial and periosteal bone. By C. W. M. Pratt. *University of Cambridge*

The histogenesis of bone at the extremities of the diaphysial tube of the mammalian long bone has received little attention and no account is given in most of the standard works. It has been suggested that the longitudinally running fibres (osteogenic fibres, fibres arciformes) of Ranvier's ossification groove (l'encoche d'ossification) are incorporated into the periosteal and perichondrial bone. A study has been made of silver-impregnated sections of this region of the femur in foetal, neonatal and growing rats. The ossification groove appears at an early stage and presents two distinct appearances. Surrounding the middle of the shaft are the trabeculae of periosteal and perichondrial bone, which have a similar finely bundled fibrillar and woven structure. At a later foetal stage when the longitudinal fibres appear in the groove they show no continuity with the bony trabeculae. The fibres of the later perichondrial bone are in fine, short and radially directed bundles. The periosteal bone becomes more coarsely bundled with age. In the neonatal period when the perichondrial bone becomes isolated in its juxta-epiphysial portion, as a ring, and in older rats when the groove gradually becomes obliterated the same essential features are maintained. These findings are discussed.

Revascularization and fate of bone transplants. By N. C. Nevin (introduced by J. J. Pritchard). *Queen's University, Belfast*

Revascularization, cell survival and new bone formation in bone transplants were studied in rats using living and boiled, autogenous and homogeneous, iliac bone inserted into lumbar spinous processes, into gluteal muscles and into the subcutaneous tissue of the ear. The vessels were injected with indian ink. In all 122 transplants were made, and these were studied histologically at intervals up to 61 days.

None of the blood vessels in the transplanted bone survived. Host vessels grew in more rapidly with living autogenous than with the homogeneous or boiled transplants, e.g. vascularization of living autogenous implants to muscle began after 7 days and was complete in 28 days, whereas in similar circumstances homogeneous bone took 42 days.

Living autogenous bone transplanted to skeletal or non-skeletal sites was rapidly replaced by new bone. Living homogeneous and boiled bone as a rule died and produced no new bone, but in three cases living homogeneous transplants to muscle did so. The first case showed new bone after 14 days, the other two after 56 days. The possibility that bone formation in the last-mentioned case was due to induction of the host connective tissues by the implant is being investigated.

The role of cortisone in relation to experimentally induced abnormalities.

By J. W. Millen and D. H. M. Woollam. *University of Cambridge*

In a series of experiments vitamin A and cortisone were administered singly and in combination to female rats of the Wistar strain during pregnancy. The vitamin A was given in doses of 60,000 i.u. by gastric intubation from the 8th to the 13th day (inclusive) and 20 mg. of cortisone acetate was injected subcutaneously daily from the 9th to the 12th day (inclusive) of pregnancy. The animals were killed on the 20th day of gestation and the young were weighed and examined for malformations. Many abnormalities were observed: only the findings concerning cleft palate and extroversion of the cerebral hemispheres were taken into account for the purposes of this communication.

It was found that there was a much higher incidence of cleft palate and extroversion of the hemispheres in those animals which had received both vitamin A and cortisone than in those which had been treated with either vitamin A or cortisone alone.

These observations suggested that in some way the administration of cortisone potentiated the teratogenic action of the hypervitaminosis A.

Observations on melanocyte distribution in freckled skin of human forearm.

By A. S. Breathnach. *St Mary's Hospital Medical School, London*

Since a freckle is a sharply localized area of pigment occurring in otherwise paler skin, freckled subjects would appear to provide ideal material for determining whether or not, in normal human epidermis, the degree of pigmentation is a function of the local concentration of melanocytes.

Cell-counts carried out upon 'Dopa'-treated epidermal spreads of forearm skin showed that there are significantly fewer 'Dopa'-positive melanocytes per unit area in a freckle, than in adjacent less highly pigmented areas, e.g., counts done on five freckles and five nearby areas of paler skin of one individual gave overall means of 951 ± 50, and 1768 ± 46 per mm.^{2}, respectively. The melanocytes of the freckles were also found to be larger and more highly active. Similar results were obtained from four other subjects.

Supra-vital staining of similar preparations with 1 in 2000 solution of methylene blue, produced no evidence that 'Dopa'-negative melanocytes were scattered among the otherwise strongly 'Dopa'-positive cells of a freckle.

These findings confirmed the conclusion of previous workers that the level of pigmentation of human epidermis is related primarily to the degree of functional activity of its melanocytes, rather than to their actual numbers.

Development and age changes of human digital Pacinian corpuscles (*Corpuscula lamellosa*).

By N. Cauna and G. Mannan, *King's College, Newcastle upon Tyne*

Fingers of 120 individuals from 71 mm. c.r. length to 93 years of age have been studied by cytological and nerve staining methods and wax plate reconstructions.

It has been found that the development of the corpuscles starts at approximately 80 mm. stage and their essential structure is completed by the sixth foetal month. During

the initial stage the receptor is cellular and its nerve fibre ramifies freely among the cells. During the intermediate stage the branches of the nerve fibre disappear, lamellae are formed from the surrounding adventitial tissue and a connexion is established with the blood circulation. During the maturation stage, lamellae and interlamellar spaces are fully formed and the central axon develops new branches which end as fibrillar swellings within the central core.

In contrast to the superficial cutaneous receptors, which are supplied from the corial nerve plexus and have to send impulses over heavily shared 'party lines', the Pacinian corpuscles receive thick medullated axons derived directly from the collateral digital nerves or their branches and so possess 'private lines'.

Post-natally the Pacinian corpuscles increase in diameter by development of new lamellae and in length by retrograde growth of the corpuscle along its nerve. Retrograde growth followed by apposition of new lamellae may result in a fusion of adjacent corpuscles. Extensive retrograde growth alone produces long tortuous corpuscles with irregular constrictions. A long corpuscle with an extensive constriction may appear as two separate units linked together by the nerve which traverses the proximal and ends in the distal corpuscle.

An experimental study of the extrinsic nerve supply of the alimentary tract in the rat. By Graeme Schofield. *University of Oxford*

Within 8 hr. following bilateral cervical vagotomy, the number of fragmented nerve fibres in the enteric plexus has reached a maximum throughout the alimentary tract. Similar changes occur throughout the gut following bilateral division of thoracic and lumbar spinal nerve roots (equivalent to interruption of the thoraco-lumbar sympathetic outflow). On the other hand, 24 hr. after either operation very few fragmented nerve fibres can be seen.

Division of either both vagus nerves or the thoracic and lumbar spinal nerve roots on both sides in the rat also results in a significant increase in the number of argyrophilic swellings in the enteric ganglia compared with those in control animals.

An estimate of the number of intraganglionic argyrophil swellings in several selected areas of stomach has been made in a series of rats, each of which has been submitted to one of the following procedures:

(1) Division of both vagi.

(2) Bilateral division of thoracic and lumbar spinal nerve roots.

(3) Division of both vagi together with bilateral division of thoracic and lumbar spinal nerve roots.

The number of swellings counted following vagotomy or sympathectomy alone is strikingly similar; the number following the combined operation is approximately double that following either operation alone.

It is concluded that the ratio of vagal to sympathetic preganglionic nerve fibres is approximately 1 to 1 in the areas of stomach selected for study.

The reconstruction of the upper limb bones of *Proconsul africanus*. By J. R. Napier and P. R. Davis. *Royal Free Hospital School of Medicine, London*

These fossilized upper limb bones, including parts of the right and left humeri, the left radius, a portion of the left ulna and a number of carpals, metacarpals and phalanges, were found in association with jaw and skull bones in a pot-hole of early Miocene age on the Gumba Peninsula on Rusinga Island in 1952.

The fossil bones are believed to belong to one individual, a juvenile of the species *Proconsul africanus* (Hopwood, *J. Linn. Soc.* (*Zool.*) *Lond.* 38, 1933). The reconstruction of the left upper limb is described and discussed.

Observations on the brachial index of *Proconsul africanus*. By J. R. Napier and P. R. Davis. *Royal Free Hospital School of Medicine, London*

The brachial index of the juvenile specimen of *Proconsul africanus* has been calculated from the length of the reconstructed left humerus and the intact left radius by applying the formula $\frac{\text{length of radius} \times 100}{\text{length of humerus}}$.

The brachial indices of living primates (both juvenile and adult) have been obtained from the data given by Schultz (*Hum. Biol.* 2, 1930), and from other specimens measured by the authors. These brachial indices have been compared with those of *P. africanus* as have also the brachial indices of several fossil specimens, particular reference being made to *Limnopithecus macinnesi* of the early Miocene of East Africa, and *Cebupithecia*, a fossil ceboid from the late Miocene of Colombia. The significance of the findings is discussed.

The fine structure of the carotid body glomus cell in the rabbit. By J. D. Lever, J. D. Boyd and P. R. Lewis. *University of Cambridge*

The glomus cells of the rabbit carotid body show considerable variation in overall electron density. The darker cells contain a background cytoplasm of noticeable electron density in which lie compact mitochondria with a content of internal cristae and filaments. In the lighter cells, however, this cytoplasmic matrix is less dense and the mitochondria are grossly vacuolated and distended.

Of particular interest has been the constant finding in these glomus cells of small ($0{\cdot}05$–$0{\cdot}15\,\mu$) osmiophile granular elements often seen to be contained by a delicate membrane. Variations in electron density within individual granules and from granule to granule are common. The population of these elements is larger in the dark than in the light glomus cell. Granules of comparable electron microscopic appearance have already been described in the rat adrenal medulla (Lever, J. D., *Endocrinology*, **57**, 1955), but these range in size from $0{\cdot}05$–$0{\cdot}4\,\mu$.

Although the exact cellular-vascular relationship is not yet clearly understood, glomus cells can frequently be seen in close approximation to capillaries. What appear to be nerve terminals have been observed in contact with glomus cells but not with capillary walls.

Following interruption of Hering's nerve, the immediate stimulation of its peripheral end produces no obvious change in the electron microscopic appearance but leads to capillary engorgement. Further experimental work is in hand.

Electron microscopy of Paneth cells in the mouse. By A. D. Hally and I. M. Dawson. *University of Glasgow*

The Paneth cells in the proximal jejunum of the mouse have been examined with the electron microscope.

The numerous large secretory granules are situated in the upper half of the cell. They are osmiophilic, spherical and homogeneous, usually with an enclosing membrane. Smaller secretory granules are in relation to the Golgi zone. Clusters of very dense particles are also found in the upper half of the cell. Each cluster is the size of a secretory granule.

The Golgi zone is supranuclear. Scattered mitochondria are present, mainly in the basal half of the cell. They are short cylinders with well-marked cristae. The nucleus is basal and is enclosed by a double membrane, in which pores can be seen.

The granular membrane pairs of the endoplasmic reticulum (α-cytomembranes) are well developed. They appear as parallel lamellae filling most of the basal part of the cell and extend upwards around the nucleus.

Compared with the brush border of the columnar epithelium of the villus, the microvilli on the free border of the Paneth cell are shorter and fewer in number.

The significance of these findings is discussed.

The distribution of glycogen in early human placentae.
By J. D. Boyd. *University of Cambridge*

The distribution of glycogen has been studied histochemically in three early human placentae and implantation sites. The associated embryos are of the four-somite stage, the thirty-somite stage and of 6 mm. C.R. length.

On the foetal side glycogen is found in large amounts in the syncytiotrophoblast, in Langhan's layer, in the cytotrophoblastic columns, in the mesoderm of the villous cores, in the lumina of the veins of the villi and in many of the blood cells in these veins. It is absent from the foetal arteries and their contained blood cells.

On the maternal side large amounts of glycogen are present in the endometrial gland cells and in the dilated gland cavities. It is also present in the decidual cells and in the maternal veins opening from the intervillous space. Except where they are plugged by cytotrophoblast the spiral arteries contain no glycogen in their lumina, though the connective tissue cells surrounding these vessels possess it. The intervillous space itself contains large amounts of stainable glycogen and, in regions where the brush border is well developed, the appearances presented suggest strongly that glycogen in particulate form is passing through the superficial syncytium into the substance of the villi.

Observations on the development of the vaginal wall.
By D. Bulmer. *University of Aberdeen*

Despite the abundance of literature on the development of the vaginal epithelium, little attention has been paid to the fibro-muscular wall of the vagina. In the present study the differentiation of the wall of the urogenital sinus and vagina has been investigated in sheep foetuses and human foetuses.

In the sheep differentiating muscle fibres are first apparent in the mesodermal condensation of the sinus at the 50 mm. stage and form a complete investment for the vestibule, with a short cranial extension around the lower vaginal segment. The vaginal muscle itself is not evident until the 115 mm. stage.

In the human foetus the sinus muscle also differentiates much earlier than the vaginal muscle. At the 112 mm. stage the mesodermal condensation surrounding the vagina consists of a dense outer layer with differentiating muscle fibres, a fairly wide intermediate layer of loosely arranged connective tissue and a narrow inner layer of dense cellular connective tissue. The external layer is continuous above with the outer layer of uterine muscle, the intermediate layer extends up for a short distance outside the inner layer of the cervical muscle, and the inner layer is continuous with the cervical mucosa. The enormous enlargement of the vaginal mass subsequently obliterates the intermediate layer, and the vaginal fornices fill its cranial extension.

These features are discussed in relation to the epithelial changes occurring in vaginal development, of which they appear to be independent.

The compound elliptical polarizer. By M. A. MacConaill. *University College, Cork*

The compound polarizer consists of a disc of polaroid, a disc of polythene and a disc of thin cellophane, mounted in that order; these three form the polarizing system, and each can be rotated. The analyser is a polaroid disc; it is held in a removable mounting which is so shaped that it fits into the nose-piece of the microscope above the objective and permits the objectives to be easily changed by swinging; it functions as a fixed analyser. The compound polarizer produces a beam of elliptically polarized light. This beam can be of any desired colour over the visible spectrum range. The elliptical polarization of the plane-polarized light produced by the polaroid is achieved by the cellophane disc. The polythene disc serves as a fine adjustment, that is, it 'gears down' the rapidity of the colour changes which could be produced by rotation of the cellophane disc and enables a critical adjustment

of the coloured beam to be easily made. The paper and demonstration show how what has been looked upon as a defect of commercial cellophane in polarizing work enables very remarkable stereochromatic effects to be produced. By means of the compound polarizer it is possible to make a 'visual dissection' of birefringent elements in tissues to be accurately carried out.

An ultramicrotome. By A. L. Sims and T. S. Leeson. *University College, Cardiff*

The development of ultra-thin sectioning has progressed rapidly in the past 6 or 7 years and many commercial machines are available but generally are expensive. This ultramicrotome is so designed that it can be made for a few pounds in a laboratory workshop where a lathe and a milling or shaping machine are available. The body of the machine is made of a metal with a very small and linear coefficient of expansion and carries both the knife and the specimen-holder. The specimen is advanced over the knife by heating the specimen-holder with a small electric heater, although alternative methods of heating are available. The knife is glass. Rotation of the specimen-holder is effected by an electric motor, sectioning being fully automatic. Contact between motor and microtome is indirect through a magnetic field which avoids vibration of the specimen during cutting. A clear visual field is provided for the operator to use a binocular microscope. Serial sections of $\frac{1}{50}\mu$ (200 Å) can be obtained readily, and their collection and orientation on a specimen grid is facilitated by *a small piece* of apparatus which can be attached to a binocular microscope.

Development of an arterial collateral circulation to the rat testis. By Mary S. Sprott (introduced by J. J. Pritchard). *Queen's University, Belfast*

The opening up of a collateral circulation has been investigated in a series of rats by following the changes which occur in the deferential artery and its branches after ligation of the internal spermatic artery. The vessels have been studied macroscopically, histologically and by injection of radio-opaque medium. The blood flow has been directly measured and the effects on the testis studied macroscopically and histologically.

It has been found that the changes in the collateral circulation occur in two phases: (1) a rapid phase, measured in minutes, during which there is dilatation of the existing vessels: and (2) a slow phase, measured in weeks, during which growth changes occur in the vessels whereby the lumen increases in size, the vessel walls increase in thickness, and tortuosity develops. These changes are associated with a marked increase in the blood flow.

An unexpected finding is that, whereas in adult animals ligation of the internal spermatic artery always produces complete testicular atrophy, in animals less than 100 days old the testis preserves its normal size and structure after ligation. Possible reasons for this are discussed.

The shape of the free end of the bovine penis. By R. R. Ashdown. *University of Bristol*

Examination of bovine penises at different stages of development suggests that a change in shape of the free end occurs during prepuberal growth. In an attempt to assess variations in shape numerically, the following ratios were calculated for each specimen:

$$(a)\ \frac{\text{Total length of free end}}{\text{Length of galea glandis}},\qquad (b)\ \frac{\text{Diameter at base of free end}}{\text{Diameter at base of galea glandis}}.$$

The results are summarized:

	No. examined	Mean ratios (a)	Mean ratios (b)
Bulls 1–4 years	12	3·61	1·64
Castrates 1–3½ years	157	3·19	1·25
Calves 1 day–3 weeks	11	2·08	1·15

Significant differences ($P < 0{\cdot}01$) were established between the three groups in all cases except for ratio (*b*) calves-castrates (in this case $P = 0{\cdot}05$). Within the castrates, these ratios were significantly related ($P < 0{\cdot}01$) to the degree of separation of the penis from its sheath (see previous communication 1956), and those with most complete separation most closely resembled in shape the penises of adult bulls. Those with little separation resembled the calf penises.

The change in shape revealed by these changing ratios can be detected by inspection. It is an increased tapering of the collum glandis and a relative shortening of the galea glandis. Both changes can be attributed to differential growth; before puberty the base of the free end grows more rapidly than the apex. This differential growth appears to be controlled by hormonal influences and the penises of castrates represent arrested stages of normal development.

The distribution of cholinesterase in the pancreas of the rat, cat and rabbit. By R. E. Coupland. *University of Leeds*

Formalin-fixed frozen sections of pancreas have been treated by the Koelle method and Coërs modification of this. Eserine and DFP have been used as inhibitors.

Cholinesterase activity is present in nerve fibres and ganglion cells, in the germinal centres of lymph nodes and in the smooth muscle cells of the larger ducts and blood vessels.

The nerve plexus is well shown. Nerve fibres run in the interlobular and intralobular connective tissue and form an interlacing network throughout the pancreas. From the general nerve net fibres may be traced to the islets of Langerhans where they divide into many fine anastomosing branches which are intimately associated with the cords of endocrine cells. All islets are richly innervated. No ganglion cells have been observed in the immediate vicinity of the islets; they do, however, exist as isolated clumps in the interlobular connective tissue.

Pacinian corpuscles are occasionally observed and show a positive reaction over the central nerve filament. The larger blood vessels show a rich nerve plexus in the adventitia and media.

The distribution of cholinesterase in the adrenal glands of the cat, rat and rabbit. By R. E. Coupland and R. L. Holmes. *University of Leeds*

Cholinesterase activity has been demonstrated in formalin-fixed frozen sections of adrenal glands by both the Koelle method and Coërs modification of this. Eserine and DFP have been used as inhibitors.

In all three animals there is strong medullary activity; a positive reaction is given by chromaffin cells, nerve fibres and groups of ganglion cells (if present). A strong reaction is also observed in the cells of the glomerular zone of the cat's adrenal cortex and in the capsule of the rat adrenal.

In all three species cholinesterase-positive nerve fibres are present in the cortex. The majority pass straight through the cortex and end in the medulla, but some fibres run tangentially in the subcapsular region before passing between groups of glomerular cells. These latter fibres may be observed running in the connective tissue septa, which separate groups of glomerular cells, and side branches are given off which appear to end in direct relationship with glomerular cortical cells. In the rat a similar, but less abundant plexus appears to exist in the reticular zone, but some of these fibres may be running towards outlying chromaffin cells.

The histochemical localization of cholinesterase in the superior cervical sympathetic ganglion. By R. S. Snell. *King's College, London*

It is now generally accepted that excitation of autonomic ganglion cells is accomplished by means of acetylcholine released when preganglionic nerve impulses reach the synapse. It is also believed that the acetylcholine is rapidly destroyed by the enzyme cholinesterase present within the ganglion. The present research is designed to investigate histochemically

the position of the enzyme within the superior cervical sympathetic ganglion and to identify the structures in which it is most concentrated.

Six cats have been used for the investigation. The superior cervical ganglion on both sides has been removed from each animal and frozen sections have been processed using a modification of the histochemical technique described by Koelle & Friedenwald (1949). The sections have been incubated with the substrate solutions at pH 4·2.

The results show that as the preganglionic nerve fibres enter the ganglion and approach the ganglion cells the concentration of cholinesterase in the nerve fibres becomes greatly increased. In addition it is noted that the preganglionic fibres outside the ganglion contain a higher concentration of cholinesterase than the postganglionic fibres leaving the ganglion. No cholinesterase is seen to be present within the ganglion cells, capsular cells or intercapsular glial cells. The significance of these findings is discussed.

JUNE 1957

The Summer Meeting of the Society for the Session 1956–57 was held on Friday and Saturday, 28 and 29 June 1957, in the Anatomy Department, University of Birmingham.

After the first morning session, owing to the large number of communications, the meeting was divided into two sections, working concurrently. The President (Prof. R. D. Lockhart), Sir Solly Zuckerman, Prof. R. Warwick, Prof. C. F. V. Smout and Prof. R. Walmsley occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers read:

The effect of cerebral lesions on oestrus in ferrets. By J. Herbert (introduced by S. Zuckerman). *University of Birmingham*

Hypothalamic lesions have been shown to modify oestrous cycles in rats and guinea-pigs by promoting either continuous oestrogenic secretion or constant dioestrus. In the ferret, such lesions are claimed to provoke premature onset of oestrus (Donovan & v. d. Werff t Bosch, 1956).

In the present investigation bilateral electrolytic lesions were made between the dates of 20 September and 13 December 1956, either in the thalamus or in adjacent areas of the brain, but not hypothalamus, of fourteen adult female ferrets. Another ten underwent 'control' operations which were identical except that an electric current was not passed. Many of the animals suffered minute cortical lesions when the calvarium was drilled.

Three of eight intact control animals had been born in the previous August; three arrived from dealers during November and two at the beginning of December. In all cases the first definite appearance of swelling of the vulva was taken as the onset of oestrus. Two of the eight controls showed incipient oestrus at the end of February, two during the first half of March and one during the second half of March. Two more came into oestrus during the first half of April, and one animal is still anoestrous (May 1957). On the basis of these observations and of previous work (Thomson, 1954, etc.) a diagnosis of premature oestrus was made in the experimental animals when vulval swelling occurred before the middle of February.

The dates of onset of oestrus in the operated animals were as follows:

Month	Number of ferrets with Electrolytic lesions	Number of ferrets with Control operations
End of November	1	1
Beginning of December	1	–
Beginning of January	–	1
Beginning of February	5	5
End of February	4	1
Beginning of March	3	–

Of the remaining two ferrets, one was killed while anoestrous in January and the other was still anoestrous at the end of April.

Thus fourteen out of twenty-four operated animals came into premature oestrus. These fourteen included animals with thalamic lesions and animals which had been subjected to control operations as defined above.

The onset of oestrus was correlated neither with the date of operation; nor with the position of the lesion; nor with the nature of the control operation; nor with the period of post-operative recovery. There was no difference in the response of animals into whose brains the electrode was simply inserted, and those in which a current was also passed.

It was concluded that cerebral lesions, including presumably hypothalamic lesions, influenced the reproductive cycle of the ferret in a non-specific manner and not in the specific way implied by Donovan and v. d. Werff t Bosch.

The effect of cerebral lesions on the sexual skin cycle of baboons.

By S. Zuckerman. *University of Birmingham*

In view of the belief that one or more specific 'sex centres' may exist in the hypothalamus, experiments were performed on adult female baboons, in order to obtain information about the possible influence of hypothalamic lesions on the menstrual rhythm. These experiments, which have not been described before, were carried out in 1934, in collaboration with Dr John F. Fulton. In the first two animals which were operated on, the lesions, which were made with a fine probe, turned out not to have been properly placed in the hypothalamus, which was approached through the corpus callosum. Nevertheless, while the menstrual rhythm was not affected, the sexual skin cycle was greatly disturbed. In a third experiment, therefore, the corpus callosum only was incised. The sexual skin cycle was again disturbed. In view of the fact that the sexual skin rhythm is disturbed by non-specific operations within the cranial cavity, it is obvious that inferences derived from observations of the effects of hypothalamic lesions at present provide little justification for ascribing the temporal control of the menstrual cycle to some specific hypothalamic factor.

The effect of surgical thyroidectomy on the induction of oestrus by light in ferrets.

By W. A. Marshall and A. P. D. Thomson. *University of Birmingham*

The present experiment was designed to find out whether or not surgical thyroidectomy interferes with the precocious oestrus which normal mature ferrets undergo when they have been exposed daily for several weeks to a few hours additional artificial light.

Nine female ferrets were thyroidectomized during July and August, 1953 and sham operations were carried out on four animals. From 3 October 1953 onwards all the operated animals together with fourteen normal ferrets, acting as controls, were exposed to artificial illumination daily between 4.30 p.m. and 10.30 p.m. in an experimental room which was fitted with 'daylight fluorescent tubes' 5 ft. long, fixed vertically to the walls between adjacent rows of cages.

Three methods were used to determine the completeness of the operation: (i) determination of the rate of oxygen consumption; (ii) estimation of the uptake of radioactive iodine in the living animal and at autopsy; and (iii) histological study, with projection and planimetry, of serial sections of the tissues in the neighbourhood of the thyroid gland.

By 28 December 1953 all the non-thyroidectomized and only two (T.F. 795 and 790) of the nine thyroidectomized ferrets had begun to show signs of oestrus. Three of the anoestrous thyroidectomized ferrets were killed on 15 January 1954, and the remainder, with the exception of T.F. 747 and 807, were still anoestrous when all the non-thyroidectomized animals were in full oestrus. (T.F. 747 showed signs of the onset of oestrus on 21 January 1954, and continued to show slight perivaginal swelling until 20 February 1954, but the animal never came into full heat. T.F. 807 also showed signs of the onset of oestrus

when examined on 17 January 1954, but the slight perivaginal swelling had disappeared four days later.)

The results of the estimations of the completeness of thyroidectomy were presented in a table. They indicated that removal of more than 95 % of thyroid tissue would delay the onset of light-induced oestrus in ferrets (T.F. 787, 796, 792, 802 and 805). They suggested that removal of 90 % of thyroid tissue might result in aberrant oestrus (T.F. 747 and 807) and that removal of 75 % of thyroid tissue might be without effect as far as the timing of the onset of light-induced oestrus was concerned.

Section of the pituitary stalk in the monkey. By E. Brodie Hughes and S. Zuckerman. *University of Birmingham*

Mature female rhesus monkeys were used to determine whether menstrual cycles continue after division of the pituitary stalk, and after the insertion of a plate of polythene to maintain the anatomical separation of the pars distalis below and the median eminence above. The approach to the pituitary stalk was by the temporal route, and the operation was carried out at varying times during the menstrual cycle. All the animals (which were studied for periods ranging from 6 to 15 months) suffered from persistent and profuse diabetes insipidus. In certain instances the volume of urine excreted daily remained at over four times the average pre-operative level for as long as a year. In practically all cases, too, a phase of uterine bleeding supervened a few days after the operation. Some animals underwent no further phase of bleeding throughout the period of post-operative observation; in others, uterine bleeding continued at normal intervals. In only one of four animals whose tissues have so far been studied histologically was the stalk completely divided and the polythene barrier still in place. In this animal, the neural process had involuted, and there were no vascular connexions between the pars distalis and the floor of the third ventricle. A wax-plate reconstruction made from projections of serial sections was exhibited to demonstrate this point. In addition to the post-operative phase of uterine bleeding, which occurred 5 days after section of the stalk, this animal underwent a further phase of bleeding 15 days later. It then remained in a state of amenorrhoea for 5 months before it was autopsied.

The effect of increasing intensities of light on the rate of appearance of light-induced oestrus in normal ferrets and in ferrets after the removal of both superior ganglia of the cervical sympathetic chain. By W. A. Marshall and A. P. D. Thomson. *University of Birmingham*

The observation by Abrams, Marshall & Thomson (1954) that removal of both superior cervical sympathetic ganglia delays the onset of light-induced oestrus in ferrets has been confirmed by Donovan and v.d. Werff t Bosch (1956). Since the operation also produces ptosis the latter authors argued that the delay was due to insufficient light reaching the retina. The following experiment was designed to test this hypothesis.

A room was partitioned off into five compartments, each of which was provided with strip lighting whose brightness increased by a factor of approximately two from about 10 f.c. in compartments A and B to about 125 f.c. in compartment E. Beginning 31 October 1956 these lights were switched on from 4 p.m. to 10 p.m. daily. At all other times the compartments were subject to the ordinary sequence of daylight. A table is produced which shows (*a*) the distribution of thirty-four normal and thirty ganglionectomized ferrets at the beginning of the experiment, and (*b*) the mean date and the range of dates of the occurrence of full oestrus of the normal and operated animals in all the compartments.

The results indicate that the rate of onset of light-induced oestrus in normal ferrets and in ferrets after ganglionectomy is not influenced by the range of light intensities explored.

The cost of maintaining the animals has been met from a grant to one of us (A.P.D.T.) from the Agricultural Research Council.

The decline of litter size with age. By D. L. Ingram, A. Mandl and S. Zuckerman. *University of Birmingham*

It is known that the number of oocytes in the mammalian ovary decreases with age. Fertility, after an initial rise immediately after the onset of reproductive life, is also believed to decrease with time. The possibility thus suggests itself that the decline in fertility is related to a depleted reserve of potential ova.

The breeding records of the colony of albino rats maintained in the Department of Anatomy, University of Birmingham, for the last 9 years (and from which animals used in a long series of oogenetic studies were derived) were subjected to statistical analysis in order to test this hypothesis.

The results first showed that the number of animals which gave birth to multiple litters rapidly falls. Thus, out of 150 females which gave birth to two litters, only eighty-five gave birth to six, and only one to twelve.

The breeding records of thirty-five animals which had eight litters were analysed separately, and the regression relating litter size to serial number of litter determined. It was found that the number of young per litter decreased in successive litters.

Age changes in the basicranial axis of primates. By E. H. Ashton. *University of Birmingham*

As seen in sagittal section, the cranial base comprises the basicranial axis extending from the basion to the prosphenion (or pituitary point), together with extensions from its anterior end to the nasion, and from its posterior end to the opisthion. The spheno-ethmoidal angle lies between the axis and its anterior extension, and the foramino-basal angle between the axis and its posterior limb.

Different regions of the cranial base show different patterns of growth, reflecting contrasts in the age changes in the endocranial cavity and facial skeleton. Differences in the growth patterns of corresponding regions in different primates can be attributed to variations in the relative size of their face and cranium.

Thus, in common with the facial skeleton, the basicranial axis and its anterior extension continue to grow until maturity, most growth occurring in those species with the biggest faces. The posterior limb, which is the antero-posterior dimension of the foramen magnum, grows in phase with the endocranial cavity, adult size being practically attained by the time the first permanent molars are in line. The spheno-ethmoidal angle, which in lower primates continues to increase until maturity, closes in man during postnatal growth. This contrast may be related to differences in the 'set' of the facial skeleton relative to the brain case. The foramino-basal angle closes in man, monkeys and apes after the first permanent molars have erupted—a change which may be associated with growth in the planum nuchale during development of the nuchal muscles.

The 'iliac horn'—an unusual pelvic anomaly. By D. Darlington. *University of Birmingham*

The gluteal region was dissected in a human subject who exhibited an inherited anomaly of the pelvis (Hawkins, *Lancet*, i, **803**, Case no. 1, 1950). A prominent spur of bone, the 'iliac horn', projected laterally from the middle of the gluteal surface of each iliac bone. The shape of the iliac crest was abnormal and the tubercle of the crest was absent. The gluteus medius took a partly tendinous origin from the sides and undersurface of the bony spur, and the sacrotuberous ligament extended laterally to be attached along its posterior margin. No other structure was attached to the spur; its superior surface was subcutaneous. The origins of the gluteal muscles were displaced, but the muscles were otherwise normal.

There was no evidence that the 'iliac horn' resulted from the traction of an abnormal muscle. It was concluded that the presence of the horn was determined by a mechanism intrinsic to the developing iliac bone.

Sex differences in the foetal pelvis. By Barbara J. Boucher (introduced by R. J. Harrison). *London Hospital Medical College*

Sex differences in the pelvis have been studied in ninety-six American Negro and thirty-three American White foetuses. The bony sciatic notch has been studied in 107 British White foetuses also. A point 'A' has been defined, from which the ischial and pubic lengths have been measured. No sex differences in the ischium pubis index, bony or cartilaginous, have been found. A large sex difference has been found in the subpubic angle. (There is no evidence that this is less marked than it is in the adult.) The width and depth of the sciatic notch, and their rate of increase with age have significant sex differences, and it is suggested that the lesser rate of increase of the width of the notch is a specific male characteristic. The sciatic notch index has been found to be significantly higher in females than in males in all groups but the American White. Racial differences, for the study of which the data are incomplete, and the discrepancy of the American White are discussed. The use of endocrinologically abnormal material to study both the sciatic notch index and its low correlation with the subpubic angle is suggested.

Observations on erythropoiesis with the electron microscope. By A. R. Muir and D. N. S. Kerr. *University of Edinburgh*

Normoblastic and megaloblastic erythropoiesis was examined in human bone marrow and in rat and rabbit embryos. Light, phase contrast and electron microscopy were employed on methacrylate-embedded tissues after fixation in osmium tetroxide or potassium permanganate or formol saline. An increase in electron density which accompanied, and was considered to be due to, the development of haemoglobin was used as the criterion of identification of the erythrocyte series. Mitochondria, endoplasmic reticulum and Golgi bodies were seen in the cytoplasm of the early erythroblast; in the later stages the mitochondria appeared, in the electron-dense cytoplasm, as clear spaces with the characteristic membrane structure. The nucleoplasm of the spherical nucleus showed the same changes in density and texture as the cytoplasm, so that in the late erythroblast the nucleus was so dense that it could still be recognized in the haemoglobin containing cytoplasm. In the circulating normoblast there was a dense mitochondrial matrix and the nucleus was frequently lobulated.

The significance of these observations in relation to the theory of nuclear extrusion was discussed.

The electron microscopy of the superior cervical ganglion after pre- and post-ganglionic section. By A. A. Barton. *Royal College of Surgeons*

The superior cervical ganglion of the rabbit has been examined using the electron microscope.

Four main groups of structures have been distinguished: (1) neurones; (2) satellite cells surrounding the neurone containing nerve fibres; (3) groups of fibres with associated Schwann cells; (4) collagen fibrils.

The changes following pre- and post-ganglionic section and after combined pre- and post-ganglionic section within 7 days of one another have been examined.

Following excision of a length of the pre-ganglionic trunk the nerve fibres within the satellite cells are present at 3 days, but at 7 days the reaction within the ganglion is so marked that structures have not yet been distinguished. Fourteen days after pre-ganglionic section the large majority of the fibres within the satellite cells disappear, and by 28 days the satellite cells seem to be separating from the neurone. There remains a residual group of fibres that persist, possibly representing connector fibres between neurones.

The groups of fibres between the neurones are clearly distinguished 14 days after section and do not appear to have diminished in number.

Fourteen days after post-ganglionic section there is a considerable increase in the number of fibre bundles between the neurones and all stages of association of nerve fibres with Schwann cells are seen.

If after post-ganglionic section the pre-ganglionic trunk is cut, the number of bundles of interneuronal fibres remains at least undiminished, whereas the fibres in the satellite cells show a marked decrease.

The effect of the growth pattern upon the structure of a long bone.

By C. W. M. Pratt. *University of Cambridge*

It has been shown that the diaphysis of the mammalian long bone grows by means of the formation of a cylinder of periosteal bone, and the incorporation of a funnel of endochondral and endosteal bone into the extremities of this cylinder. The effect of this pattern of growth on bone structure is not known, and the present investigation, based on a histological study of the femur of the postnatal rat, is an attempt to elucidate it.

In the neonatal period the diaphysis has a fine cancellous structure. The trabeculae have a woven and coarsely bundled matrix, and contain remains of the more finely bundled foetal bone. When the metaphysis forms it consists largely of perichondrial and endochondral bone. The diaphysis remains cancellous, but the matrix is parallel-fibred and finely bundled.

Subperiosteal remodelling of the subepiphysial bone follows and part of the secondary spongiosa is incorporated into the diaphysis. This endochondral bone has a fine-woven fibrous structure. This then becomes consolidated by the appearance of parallel-fibred finely bundled bone, which in turn may be replaced later by lamellar bone.

Deposition of subperiosteal lamellar bone occurs over the consolidated endochondral bone at the base of the metaphysis. As the bone lengthens and as endosteal erosion occurs this lamellar bone comes to form a large portion of the diaphysis. The portion of the diaphysis that corresponds to the neonatal shaft may retain its original non-lamellar structure.

Variations in bone structure and the value of Young's Modulus.

By R. Walmsley and J. W. Smith. *St Salvator's College, University of St Andrews*

The value of Young's Modulus for bone is known to vary considerably. These values not only vary in different bones of the same animal but also in different samples from the same bone. The present investigation was confined to the radius of the horse and the metacarpus of the ox. The central four-fifths of the cortex of the shafts of these bones were cut longitudinally into a number of rods which were then trimmed to a uniform rectangular cross-section.

It was found that the value of Young's Modulus varied considerably both along the length of a bone and around its circumference and that the variations constituted definite patterns peculiar to each bone.

An attempt has been made to correlate the variations in Young's Modulus with certain histological features of the bones. In regions of bone with different Young's Moduli the histological appearance apparently varies at two levels, in the structure of individual osteones and in the pattern of osteones throughout the region.

Elastic after-effect, plasticity and fatigue in bone.

By J. W. Smith and R. Walmsley. *St Salvator's College, University of St Andrews*

The studies of the elastic properties of bone published in recent years have concentrated mainly on its reaction to single momentary stresses. In the present investigation rods of rectangular cross-section have been prepared from the radius bone of the horse and the metacarpus of the ox. Using such rods as cantilever beams the reactions of bone to repeated momentary stresses and also to single stresses of longer duration have been studied.

When a constant stress is maintained for an appreciable period and then removed, both the development of strain and its subsequent diminution occur asymptotically; this is an example of 'elastic after-effect'. Furthermore when stresses above a certain value are applied, a degree of plastic flow occurs while the stress is maintained; this plastic flow is superimposed on the elastic after-effect. As a result, when a stress of this magnitude is removed, the rod of bone remains permanently distorted.

The repetition of stresses of a value well below the ultimate strength of bone ultimately causes a fatigue fracture, and the number of stress applications necessary to cause fracture is an inverse function of the magnitude of the stress.

Observations on the human 'carrying angle'. By F. L. D. Steel and J. D. W. Tomlinson. *London Hospital Medical College*

The 'carrying angle' has been redefined as that lying between one line, tangential to the medial surface of the articular head of the humerus and produced through the tip of the medial epicondyle, and another joining the tip of the epicondyle to the lower end of the medial border of the ulna. Previous measurements made with hinged boards brought into apposition with the soft tissues introduced an ambiguity that is eliminated by using bony landmarks. A population of 100 young adults of both sexes has been investigated. The two distal points, being subcutaneous, have been marked on cardboard with a rectangular straight edge and pointer; the medial border of the humeral head has been photographed on to a film (attached to the card) by a perpendicular X-ray beam. The means for the male and female groups are 19·28° and 18·38°, respectively; the difference between these figures is not significant statistically and no evidence has appeared to demonstrate that the size of the carrying angle is a secondary sexual characteristic.

Vascular patterns in human long bones. By M. Brookes. *University of Liverpool*

The lower extremities of twelve dead foetuses varying in C.R. length from 16 to 35·5 cm. were injected intra-arterially with 'Micropaque'. The vascularization of certain skeletal elements was then studied radiologically.

Radiographs of foetal femora and tibiae showed that the cartilaginous primordia of the extremities of these bones are well supplied with arteries, whose pattern is similar to that of the smaller arteries contained in adult bony epiphyses. In particular, the cartilaginous femoral head was arterialized in all specimens by vessels entering at its rim, at first predominantly from the anterior aspect. At 22 cm. C.R. length the head received arteries throughout its circumference and through the ligamentum teres. Subsequently, two circumferential arterial groups were distinguishable, a postero-superior and an extensive inferior. Foveal arteries provided only a minor contribution to the vascularization of the foetal femoral head. Although the vascular patterns in a cartilaginous epiphysis and an adjacent bony metaphysis were distinct, an occasional vessel united the two systems. Transverse microradiography failed to reveal a periosteal arterialization of foetal bone cortex, which was constantly supplied by centrifugal branches of the medullary arterial system.

Angiography of eight tibiae obtained from adult limbs amputated for advanced arteriosclerosis with peripheral gangrene showed a predominantly periosteal arterialization of bone cortex. Cortical veins were profuse, generally radiating from a central venous channel. In the lower third of the tibia, however, the venous pattern changed abruptly to a longitudinal one. It was suggested that periosteal arterialization of bone cortex was a pathological phenomenon.

Observations on glycogen in relation to the blood vessels of the human placenta and uterus. By J. D. Boyd. *University of Cambridge*

Histochemical studies on early human placentae have led to observations on the distribution of glycogen in the walls of the blood vessels of the chorionic villi and umbilical cord and of the arteries and veins of the decidua. The pattern and arrangement of the glycogen in these sites is described. In addition attention is drawn to the large amounts of particulate glycogen which can be identified, by the periodic acid Schiff reaction, in the lumina of the foetal vessels of the chorionic villi and the umbilical cord. Glycogen can also be identified in the uterine veins and in the cellular plugs (Boyd & Hamilton, *J. Anat., Lond.*, **90**, 595, 1956), which are found in the decidual spiral arteries. Histological observation alone cannot establish definitely that the intravascular glycogen is not a fixation artefact. The regularity of its presence, however, and a detailed study of its distribution suggest that in the stages of gestation concerned glycogen may normally be present in part of the foetal blood stream.

A chimpanzee placenta and foetus *in situ*. By W. R. M. Morton. *Department of Anatomy, Queen's University, Belfast*

The pregnant uterus and its contents from an adult chimpanzee (*Pan satyrus* L.), aet. 9 years, was described. The contained female foetus of 25 weeks ovulation age (approx.) was presenting in the left occipito-posterior position, and after fixation weighed 1060 g., and measured 243 mm. in sitting height. The placenta, a single almost circular disc, measured 118×100 mm. in its main diameters, and 19 mm. in thickness at the umbilical cord. It was firmly attached to the dorsal wall and fundus of the uterus, and overlapped the left tubo-uterine opening. The 33·5 cm. long umbilical cord was attached eccentrically a little below and medial to the midpoint of the placenta. The non-placental chorion was firmly attached to the uterine wall, except below where it lay over the dilated cervical canal. This canal contained dilated gland-openings visible on inspection, and the patulous external os protruded into the vagina for approximately 2 cm. distal to the posterior fornix. The microscopical appearances of the haemochorial placenta resembled those of a 230 mm. c.r. human foetus of 7 months. Some villi were small, contained a highly cellular mesenchyme and conspicuous foetal capillaries; others formed large lobulated masses which were more obvious than in the human. Fibrinoid was present in considerable amounts in the basal plate and villous regions, but formed only a thin layer in the chorionic plate. Decidual cells were present, and mono-nuclear 'giant' cells were present in the deeper layers of the decidua. The maternal ovaries were small and inconspicuous.

***In ovo* technique for operating on the early chick embryo.** By P. H. S. Silver. *Middlesex Hospital Medical School, London*

Hitherto, experiments on the early chick embryo during the first 36 hr. of incubation have been fraught with the greatest difficulty. A series of 600 experiments have been performed, in which a clear picture of the causes of death has been revealed and a technique devised which allows normal development to take place.

During the early stages the blastoderm is covered by the tense vitelline membrane. An opening has to be made in this membrane to gain access to the underlying blastoderm. Subsequently, the embryo or blastoderm tends to bulge through this hole and to become adherent to its edges. The level of the adhesion is governed not only by the position of the opening, but also by the fact that, as they develop, the head, neck and trunk regions migrate forwards in relation to the vitelline membrane. Thus, an opening made over the midbrain at the six-somite stage may adhere next day to the medullary folds opposite the twentieth somite, producing spina bifida, scoliosis, etc. and preventing amniotic development. Adhesion of the blastoderm lateral to the embryo prevents the development of the area vasculosa.

The vitelline membrane is normally separated from the shell membrane by a thin layer of albumen. If this albumen is allowed to dry, or is removed during the experimental procedures, adhesion will occur between the vitelline and shell membranes and the development of the embryo and spreading of the area vasculosa may be abnormal.

The following technique has been employed:

(1) The vitelline membrane is repaired using a single silk thread. It is necessary first to remove a small quantity of yolk so as to reduce tension in the membrane. The edges of the opening are then picked up with blunt-nosed forceps and a ligature tied.

(2) Drying is prevented by irrigating with watery albumen and not saline.

(3) The egg is topped up, the window replaced, and the egg rolled 180°.

Nerves of the rodent heart and ductus arteriosus.

By R. L. Holmes. *University of Leeds*

Techniques for the demonstration of cholinesterase activity have been employed to show nervous structures in the heart wall and ductus arteriosus of rabbits and guinea-pigs. The atrial wall has an extremely rich innervation. This is in the form of a fine-meshed network which receives contributions from bundles of thicker nerve fibres. Terminal ('receptor') structures such as those readily demonstrable in the hearts of cats and dogs have not been observed in these preparations.

A plexus of nerve fibres lies in the wall of the ductus arteriosus, and is especially prominent in the foetal rabbit. This close association of nervous elements with the ductus persists into adult life. The fibres are readily apparent in whole-thickness preparations from foetal animals on account of low cholinesterase activity in other tissues of the ductus wall, which contrasts with a stronger reaction found in the walls of the pulmonary and aortic trunks. The significance of these observations in relation to function is discussed.

Reduplication of bladder and associated anomalies—report of a case.

By N. F. Kirkman and G. T. Ashley.

A female newborn infant, with a congenital umbilical hernia, was seen and operated on by one of us (N. F. K.). Anomalies of pelvic viscera were suspected at the time of operation. The child survived for 6 months, then contracted pneumonia and died. The pelvic viscera and perineum were removed *en bloc* and sent to a pathologist for report. After sundry incisions had been made into rectum, vagina, uterus, bladder and perineum, the specimen was sent to one of us (G. T. A.), for further investigation. The findings of this investigation were reported and illustrated.

The intrinsic blood vessels of the pelvic colon.

By J. G. Brockis and D. B. Moffat. *The Royal Infirmary and University College, Cardiff*

The blood vessels of the wall of the pelvic colon have been studied by the injection of Neoprene latex 572 into the arteries and veins of specimens obtained post-mortem.

The arteria recta, which at first lie in the subserous layer, pierce the muscular coat at various levels between the mesocolic attachment and the distal taeniae. Whilst in the subserosa they supply the appendices epiploicae and give numerous fine lateral branches which, together with those of the neighbouring arteria recta and perforating vessels from the submucosa, form an external muscular plexus.

Having reached the submucous layer, the main arteries split up, some of their branches forming a series of arcades with neighbouring arteria recta, while others pass towards the antimesenteric border where they are continuous with the vessels of the opposite side. From this main submucous plexus branches run towards the mucosa, anastomosing with similar neighbouring branches to form a secondary plexus which gives rise to the mucosal

arteries. These pierce the muscularis mucosae and form a plexus on its internal surface from which the mucosal capillaries arise.

The main submucous plexus also gives rise to recurrent branches which pass to the deep surface of the muscle coat to form an internal muscular plexus. Many of these branches perforate the muscle to join the external muscular plexus.

In general, the veins follow the course of the arteries. Some surgical applications of these findings are discussed.

Nerve fibre differentiation in submucous plexuses.

By A. D. Dixon. *University of Manchester*

In a previous communication the morphological features of nerve endings and submucous nerve plexuses in mammalian oral mucosa were described. The topographical features of the plexuses can be demonstrated readily by routine nervous-tissue staining techniques, but it is more difficult to differentiate between somatic and autonomic nerve fibres. In silver preparations, for example, this is due to differences in the degree of staining of serial frozen sections and artefacts, so that the distinction between myelinated and non-myelinated fibres is masked.

An osmium tetroxide staining method (Champy, 1913), reputedly specific for adrenalin-like substances, has been employed in the present study to stain autonomic fibres constantly and selectively. It has been found that not only are postganglionic autonomic fibres demonstrated as a succession of tiny black beads or fine lines but, in addition, the somatic fibres also stain, showing well defined myelin sheaths with nodes of Ranvier.

The fibre bundles composing the submucous plexuses are seen to consist of a mixture of both somatic and autonomic fibres, the latter contributing to extensive perivascular nets and a terminal network in the submucous connective tissue.

The nature of the differentially stained fibres, as revealed by the apparently general nerve stain, are a constant feature and have been confirmed by animal experiments. Preliminary results indicate an extensive autonomic anastomosis across the midline, a finding which is being investigated more fully.

The comparative morphology of the myenteric nerve plexus.

By J. R. Rintoul. *University of Manchester*

A variety of vertebrate animals has been employed in the study of the myenteric plexus. It is found that the arrangement of the fibre tracts and ganglia forms a pattern which is distinctive for any one species of animal, but which differs considerably for animals of different species. The form of the myenteric plexus is also seen to differ considerably in different parts of the guts of the same animal. In this communication attention is mainly confined to the pattern in the small intestine.

It is shown that each species of animal possesses a characteristic key pattern which can sometimes be reduced to a simple geometrical figure, e.g.

(1) The rat shows typically a plexus of close mesh composed of rectangles.
(2) The rabbit has a wide meshwork of six- or seven-sided figures.
(3) The monkey possesses a meshwork built up from pentagons.
(4) The pigeon possesses a loose meshwork in which the meshes are fusiform in outline.

Photomicrographs of the preparations were shown, the magnification in each case being the same, so that a true comparison could be made.

The effect of using different nerve stains and fixatives has been investigated. It is found that the pattern remains essentially the same whatever method is employed—methylene blue, modified Bielschowsky or Champy-Coujard techniques.

Electron microscopic observations on the myenteric plexuses with special reference to smooth muscle innervation. By K. C. Richardson. *University College, London*

The interstices of Auerbach's plexus in a whole mount of intestinal longitudinal muscle show secondary and tertiary nerve bundles issuing from primary bundles and ganglia, together with capillaries and numerous stellate cells whose long processes intermingle to form a network. As described by Taxi (*Arch. Anat. micr.* **41**, 1952), the latter network is distinct from that produced by tertiary nerve bundles, and corresponds to the interstitial cell network revealed by Golgi or methylene-blue staining. Staining methods for peripheral nervous tissue commonly show one or other of these networks, but not both simultaneously.

Examination of the muscle coats and plexus tissue in the rabbit small intestine by electron microscopy has established that:

(*a*) The neurones and nerve fibres of Auerbach's plexus are ensheathed by the cytoplasm and membranes of cells comparable with the Schwann cells covering non-myelinated fibres elsewhere.

(*b*) This covering, extending over the secondary and tertiary bundles, including their branches ramifying within the muscle, contains individual nerve fibres rather than a tangled assortment of neuro-fibrils or terminal reticulum. The intramuscular bundles show fibres emerging at the surface of the sheath, sometimes leaving it altogether, and coming in close apposition with the muscle membranes. Some of these supposed nerve endings and some of the fibres in the bundles contain synaptic vesicles.

(*c*) The stellate interstitial cells lie surrounded by connective tissue in the interval between the two muscle coats and make random contacts with the nervous, vascular and muscular components. They accompany the intramuscular nerves but do not achieve a more extensive distribution in, nor a more intimate relationship with, the muscle than the nerve fibres. The interstitial cells contain more ergastoplasm than do Schwann cells, and no fibrillar component comparable with nerve fibres in Schwann cells. They appear to be fibroblasts modified in shape by the minute spaces in which they exist.

The development of regional variation in the frequency distribution of skin appendages of the human integument. By George Szabo. *London Hospital Medical College*

The frequency distribution of sweat ducts and hair follicles has been estimated in foetal, infant, adult and senile human skin, by estimating the number of these appendages in 'split skin' preparations. It has been found that there is a great regional variation in the adult, as there are about 800 appendages per cm^2 in the skin of the head, whereas their number varies in the rest of the body between 180 and 250. The variation is, however, small in the infant skin and even smaller in the foetus. Calculations have shown that the regional differences in the growth rate of the body are responsible for the regional variation in the distribution of skin appendages. Initially the appendages develop in equal number per unit area; during the postnatal development, however, they become spaced out in the extremities and in the trunk to a greater extent than on the head. It is postulated, therefore, that neogenesis of hair follicles or sweat ducts does not take place in the normal adult skin, i.e. hairs are replaced during the hair cycle but their absolute number does not increase.

The lymphatic drainage of grafted skin. By R. J. Scothorne. *University of Glasgow*

The re-establishment of lymphatic drainage from grafted skin has been studied, in the rabbit and guinea-pig, by three methods:

(1) Injection of lymphatics with indian ink at intervals after grafting.

(2) Tattooing of the graft, before transplantation, by intradermal injection of aqueous colloidal graphite; and

(3) Intradermal injection of radio-iodinated human serum albumen at intervals after grafting.

The results indicate that there are no essential differences in the rate of re-establishment of lymphatic drainage of autografts and homografts of skin. This conclusion is discussed in relation to the general theory of homograft rejection.

Gubernacular changes in the pig during and after testicular descent. By K. M. Backhouse (*Charing Cross Hospital Medical School, London*) and H. Butler (*University of Khartoum*)

The gubernacular apparatus in the pig is considered to be a mass of mesenchyme into which the processus vaginalis grows and in which the cremaster muscle differentiates. Part of the gubernacular mass is suspended in the peritoneal cavity by a mesentery extending from the caudal pole of the testis to the blind end of the processus vaginalis, the *plica gubernaculi*. The remainder surrounds the processus vaginalis and is divided into: (*a*) the *extra-vaginal portion*, surrounding and co-extensive with the processus vaginalis, and (*b*) a globular mass distal to the blind end of the processus vaginalis, the *infra-vaginal portion*.

Prior to testicular descent, the globular tip of the gubernacular apparatus comes to lie free in the scrotal sac. As the time of descent approaches (190–200 mm. c.r. length) the tip becomes withdrawn, on an average, about 5 mm. from the scrotal floor and the plica gubernaculi and the globular infra-vaginal tip become more swollen. Relatively, the greatest amount of swelling occurs in the inguinal canal where, until now, the plica has been thinnest. Thus, the testis comes to lie at the apex of an elongated cone, being less in diameter than the immediately subjacent gubernaculum. Rapid descent through the inguinal canal then occurs.

The plica gubernaculi is gradually shortened and finally resorbed as testicular descent continues, but the extra-vaginal portion of the gubernaculum is converted into fibrous tissue, containing the cremaster muscle in its substance and thus forming the spermatic fasciae.

The abundant ground substance of the gubernaculum contains much acid muco-polysaccharide material, a large proportion of which appears to be hyaluronic acid or hyaluronates. The change in bulk of this tissue is almost entirely due to increased amount of ground substance. As the extra-vaginal portion is converted into fibrous tissue the acid muco-polysaccharide containing ground substance is much reduced as its character changes; it must be remembered, however, that at this stage all foetal connective tissues show strong positive reactions for acid muco-polysaccharide.

Therefore, changes in the gubernaculum, during testicular descent, would appear to consist, to a considerable extent, of chemical changes in its ground substance, and are probably related to the effects of steroid hormones upon the acid muco-polysaccharides.

Histochemical investigations on the stellate reticulum of human foetal teeth. By A. R. ten Cate. *London Hospital Medical College*

Access to a supply of fresh human foetuses removed at operation, and dissection of the tooth germs from their bony environment, has made possible the preparation of undecalcified sections of developing teeth (up to the stage where the first increments of enamel have formed) to which histochemical techniques may be applied.

It has been found that the stellate reticulum is histochemically active from the time of its histodifferentiation. Intracellular alkaline phosphatase activity is present from the outset. In fact, it is in this localization that the enzyme first appears in any of the dental tissues. Glycogen is abundant in the stellate reticulum, occurring as fine droplets evenly distributed throughout the cytoplasm. The ground substance is metachromatic, faintly PAS-positive, hyaluronidase-labile, and gives a positive reaction with Alcian blue, all indicative of the presence of an acid muco-polysaccharide.

A variety of functions has been attributed to the stellate reticulum. They fall into two classes: (*a*) nutrition of the cells of the internal enamel epithelium and (*b*) mechanical functions. Butler (*Biol. Rev.* **31**, 1956) considered the stellate reticulum to be a tissue specialized for the imbibition of water and indirectly related to the determination of crown pattern. Histochemical findings support this view. The early appearance of alkaline phosphatase, correlated with the presence of abundant glycogen, suggests that the prime function of the stellate reticulum is the elaboration of the demonstrated acid mucopolysaccharide, bestowing upon it the property of water imbibition.

Observations on the submicroscopic structure of enamel matrix in the developing teeth of young rats. By R. W. Fearnhead. *London Hospital Medical College*

Whole molar tooth germs from 1–5-day-old rats, and the basal end of the lower incisors from 3-week-old rats were fixed either in buffered osmium tetroxide or a mixture containing 3 % potassium dichromate and 2 % lanthanum nitrate (Dalton, *Int. Rev. Cytol.* **2**, 1953). Tissues were embedded in methyl-methacrylate without the addition of butyl-methacrylate in order to produce a block hardness as near as possible to the hardness of the tissues. Sections (200–300 Å thick) were cut without decalcification using a diamond knife mounted on a rotary microtome specially constructed for cutting ultra-thin sections. The diamond knives were prepared from South African boarts by Dr J. F. H. Custers and the late Dr P. Grodzinski. The structures seen in low and medium power electron micrographs of forming enamel matrix could not be correlated precisely with the appearance of this tissue when viewed with the optical microscope. The electron micrographs show that the organic matrix of the interprismatic region is formed in advance of the enamel prism. This helps to provide an explanation of the 'picket fence' appearance seen with the optical microscope and which previously was thought to be due to the eccentric transformation of Tomes's process of the ameloblast into pre-enamel.

The responses of the 'sterile' horn of the rat uterus after certain experimental procedures. By A. Young. *Department of Anatomy, Glasgow University*

Previous communications reported internal (transuterine) migration of fertilized rat ova from the normal horn into the 'sterile' horn produced by ligation of one Fallopian tube prior to a normal mating (Young, 1954), and contrasted the effects of unilateral ovariectomy and unilateral tubal ligation on a subsequent pregnancy (Young, 1954). Further experimental procedures also showed differences in the effect on the rat uterus of these two forms of operative interference. The findings were discussed and a possible explanation offered.

A correlation of the normal development of the infra-umbilical portion of the abdominal wall with the congenital malformations occurring in this region. By T. W. Glenister. *Charing Cross Hospital Medical School, London*

In the course of correlating embryological findings regarding the normal development of the lower part of the urogenital tract with maldevelopment in the region, it has been noted that no entirely satisfactory explanation has been given, so far, concerning the development of the infra-umbilical abdominal wall.

A satisfactory explanation of normal and abnormal development in this region is to be found in the fact that the caudal end of the primitive streak gives rise to the mesenchymatous basis of the infra-umbilical abdominal wall, whereas the muscles of the region develop from mesoderm derived from the lower thoracic segments.

Some observations on the epithelial covering of the human umbilical cord. By P. Bacsich. *University of Glasgow*

The epithelial covering of human umbilical cords from the early stages of development to full term has been examined. The findings show that, unlike the epithelium of the amnion proper, which never progresses beyond the stage of a single layer of cubical cells, the epithelium of the cord undergoes with advancing age a certain degree of differentiation and maturation. These changes are qualitatively the same as those taking place in the epidermis of the body ectoderm, but start at a later date, proceed at a slower rate and never reach the same quantitative levels. While there are slight individual variations in the onset of this maturation process, by the fourth month there is invariably a distinct layer of squamous epithelial cells of different tinctorial behaviour above the original basal layer of cubical cells. These cells appear to be identical with the periderm (epitrichium) layer of the epidermis of the embryonic body. In some instances this maturation process stops at this stage, while in other cases towards the later stages of pregnancy there may be clear indications of a stratum intermedium also.

On this topic most of the present day textbooks of embryology give misleading information in words, or by inference, or through omission. Earlier workers (Minot, 1892; Lange, 1893), have already recognized the true state of affairs and have suggested that the epithelium of the umbilical cord should be classed with the body, and not with the amniotic, ectoderm. Probably it is safer to say that it occupies an intermediate position between the two.

Trans-neuronal degeneration in the specific sensory nucleus of the thalamus. By David Bowsher. *University of Liverpool*

The thalami of five human cases following antero-lateral tractotomy for the relief of pain have been examined by the method of Nissl. These thalami come from cases dying between 17 and 50 days after operation. Changes are found in a small number of cells in the specific sensory nucleus (n. ventralis postero-lateralis) of the thalamus. The abnormalities consist of diminution in cell size, accompanied by hypochromia and sometimes blurring of the outline of the nucleolus, rarely by extrusion of the nucleus. The proportion of cells affected is roughly consistent with the percentage of cortical points which give rise to painful sensations when stimulated in conscious patients (Penfield & Boldrey, *Brain*, **60**, 1937). It is, therefore, tentatively suggested that there may exist dissociation of sensation in this thalamic nucleus, and that acute localized pain is consciously represented in the cortex rather than at thalamic level.

The significance of the findings, and the general problem of trans-neuronal degeneration are discussed.

Observations on the Marchi method. By E. J. Field. *University of Bristol*

On looking through sections of normal rat or chicken sciatic nerve impregnated by the Poirier, Ayotte and Gautier modification of Marchi's method, a number of apparently normal nerve fibres are seen showing localized blackened segments along their course. For the most part they are situated near the periphery of nerve bundles. On cross-section the appearances are identical with those described by Kinnier-Wilson and Mellanby as indicating nerve degeneration in beri-beri and vitamin A deficiency, respectively.

The blackened segments pass over at either end into normal yellow-staining nerve. They are often rather swollen and show black ring-like bodies, many of which may be Elzholz bodies. The interspersal of blackened segments with normal fibres argues against their being technical artefacts, but suggests that the blackenings correspond with some real localized variations in the character of the myelin.

When sciatic nerves are cut and examined at intervals black myelin ellipsoids are present at 45 hr., but a 25μ frozen section of degenerating nerve does not blacken when

impregnated. Frozen sections, however, show a gradually increasing amount of Marchi-positive granular material within Schwann cells. Attention is drawn to the non-uniformity of staining of myelin globules in fully degenerating pieces of nerve which have been impregnated as a block. Further experiment shows that freezing either before or after fixation in formol prevents Marchi staining. Similarly, it prevents the appearance of blackened segments on normal nerves, i.e. these blackened segments behave with respect to freezing as if they were regions of frankly degenerating myelin. On the other hand, freezing does not materially interfere with staining of normal myelin by 1 % osmic acid.

The significance of these results is discussed. It is concluded that (*a*) the physical as well as the chemical state of myelin is important in determining its osmophilia, (*b*) there may be localized regional variations in the state of myelin along a nerve.

Experimental destruction of the paratympanic organ in pigeons. By J. H. F. Silver (introduced by D. R. Dow). *Queen's College, Dundee*

The paratympanic organ is a small epithelial vesicle in the middle ear of birds. It appears to be a special sense organ of unknown function. Its sensory area has hair cells and receives a branch from the facial nerve.

Vitali (1915) destroyed the organ in pigeons and reported that the birds developed wing drooping, worse on the operated side. This sometimes progressed until they were unable to fly. He found degenerated nerve fibres and cells in the vestibular system and cerebellum. Benjamins (1926) repeated the experiment with negative findings. The present work was undertaken in an attempt to clarify these conflicting results. A slight modification of Benjamins's technique was used, the organ being destroyed by electrocautery in fourteen pigeons (eleven bilaterally, and three unilaterally) and the destruction later verified histologically. One developed labyrinthitis. Of the remaining thirteen, ten behaved normally, but in three there occurred wing drooping, and in these it was found that a more extensive lesion had been inflicted.

A further series of operations was then performed precisely according to Vitali's directions. This was a more damaging operation than that devised by Benjamins and was found to produce wing drooping consistently. Thus, simple destruction of the paratympanic organ causes no obvious disability, but Vitali's operation causes wing drooping by damaging some other structure, possibly the labyrinth or the vestibular nerve.

The structure and development of the paratympanic organ are also discussed.

The dependence of the blood supply of the spinal cord on certain aortic segments. By J. B. D. Torr. *University of Manchester*

In a recent communication the blood supply of the spinal cord in the foetus has been described, and it has been noted that the arrangement of the vessels during the latter half of prenatal development resembles closely that of the adult.

Segmentally restricted injections have been made into the aorta in an attempt to evaluate the relative importance of the supplies from the vertebral arteries through their anterior spinal or radicular branches, from the upper and midthoracic radicular branches and from the lower thoracic and lumbar radicular branches. In most cases the supply from the vertebral arteries is restricted to the cervical and upper thoracic regions of the cord, whereas the supply from the lower thoracic and lumbar radicular vessels can extend to the greater part of the cord.

In many cases the supply from the midthoracic radicular vessels appears to be insignificant.

These vascular arrangements explain why certain neurological complications may follow segmental resections of the aorta.

Observations on premature fusion of the sutures of the cranial vault.
By B. H. Dawson and D. A. N. Hoyte. *University of Manchester*

The clinical and radiological features of a series of five children with premature fusion of all the sutures of the cranial vault have been examined.

When all these sutures are prematurely obliterated growth of the cranial vault becomes restricted in all directions, so that there is not enough room for the growing brain. The intracranial tension increases and marked convolutional impressions are seen in the radiographs. The cranial contour does not become abnormal as in the cases of obliteration of a single suture.

Examination of radiographs at different ages shows that in some of the cases the vault sutures cease to function at the same time, whilst in others the process of the sutural obliteration occurs in one suture after another. In some, the area of relative weakness presented by the anterior fontanelle bulges. The base of the skull is not affected.

The peripheral connexions between the lingual and hypoglossal nerves.
By M. J. T. Fitzgerald and M. E. Law. *University College, Cork*

Peripheral connexions between the lingual and hypoglossal nerve were studied in cat, dog, rabbit and pig; and in forty instances in man. In all these forms, the hypoglossal nerve forked into lateral and medial divisions, each of which made plexiform connexions with branches of the lingual nerve. One of these plexuses was extramuscular, the other intramuscular.

These connexions were studied experimentally in the pig, following an examination of the normal neurohistology of its tongue; five animals were operated on. Section of the lingual nerve caused the complete degeneration of sensory fibres in the endoglossal branches from the plexuses to the anterior two-thirds of the tongue, which fibres were unaffected by section of the hypoglossal trunk. The distribution of the sensory branches so demonstrated was to endings in the intramuscular connective tissue and in the adventitia of arterioles. Neither muscle spindles nor spiral endings were found.

It appeared that the lingual-hypoglossal plexuses were a mechanism of transfer of proprioceptive fibres from the lingual to the hypoglossal nerve for the innervation of the anterior two-thirds of the tongue.

Studies on guinea-pig peritoneal exudate cells *in vitro*.
By F. Jacoby. *University College, Cardiff*

Cell suspensions have been obtained from Tyrode washings of the abdominal cavities of guinea-pigs 3 days after an intraperitoneal injection of liquid paraffin. They have been cultured in Carrel flasks (2–3 million cells/flask) in a mixture of dilute guinea-pig and horse serum. The small percentage of granular leucocytes present perish very quickly. Fibroblastic cells multiply mitotically and may gradually form proper colonies. The bulk of the original cell population, however, consists of highly amoeboid cells, usually referred to as macrophages. These form the main object of the present studies, which are primarily concerned with their long-term cultivation and serial photographic recordings (one frame every 6 min.) of their behaviour over long stretches of time. It is possible to maintain these cells *in vitro* up to 8 weeks. Some remain small amoeboid forms, others increase in size and extend most adventurous pseudopodia. The process of clasmatosis is not uncommon and is discussed. Both forms ingest fine coal particles avidly, but do not readily segregate trypan blue, when this is added to the medium in solution in a final concentration of 1/2000. The serial photography, in some instances extending over 3 days, shows an almost complete lack of mitotic cell divisions. Thus, these cells differ markedly in several respects from similarly cultured fowl macrophages, with which they are compared.

Connective tissue formation and alkaline phosphatase. By F. R. Johnson and R. M. H. McMinn. *University of Sheffield*

Although a number of workers have considered that alkaline phosphatase is concerned in the formation of collagenous tissue, others have claimed that the association of phosphatase with young fibroblasts is an artefact produced by an affinity of these cells for the enzyme or for the reaction products formed during the histochemical procedure.

In a previous investigation on wound healing in the gall bladder of the cat, the present authors were unable to demonstrate phosphatase in new connective tissue. Lest this should be a peculiarity of this organ, connective tissue growth in the cat was studied in other organs (skin, oesophagus, small and large intestines and urinary bladder) during the healing of artificial ulcers, and in the sheath of the rectus abdominis muscle following the implantation of various tissues and substances.

In the connective tissue in all these sites, a negative reaction for alkaline phosphatase was given, even following prolonged incubation periods, except in the tissue surrounding implants of urinary bladder and gall-bladder epithelium. Since the association of phosphatase with young connective tissue was based mainly on studies of the skin of rodents, some experiments were also carried out on rats and guinea-pigs; these confirmed the positive reaction in the cutaneous connective tissue in these animals, and also demonstrated conclusively that the reaction was not due to an affinity of fibroblasts for the enzyme or reaction products. The significance of these findings was discussed.

Freeze-substitution in cytology and cytochemistry. By N. M. Hancox. *University of Liverpool*

For much critical cytological and cytochemical work, freeze-drying is the method of choice. This technique, however, possesses several practical disadvantages, among which is the necessity for a high-vacuum apparatus.

Simpson (1941) 'quenched' tissue samples as for freeze-drying. He then removed the ice, not by evaporation *in vacuo* at low temperature, but by dissolving it in organic solvents at low temperature; he called this simpler technique 'freeze-substitution'. Others using the method since, obtained cytological results indistinguishable from freeze-drying, but disagreed as to whether the ice solvent 'fixes' the tissue chemically. This question was investigated in a preliminary way as follows.

Samples of various organs were 'quenched' in isopentane chilled with liquid N_2 and transferred quickly to n-butyl alcohol maintained at -38° C. in a refrigeration apparatus. When dehydrated (2–3 days), they were transferred to an n-butyl alcohol-wax mixture and finally embedded in wax. Adjacent sections from the ribbons were 'floated-out' in order over water, physiological saline, 'Intradex' and 80 % ethanol. These reagents penetrated to the sectioned tissue. The sections were pressed down on slides and stained; the effects of the different 'flotants' were then compared.

Alcohol as a 'flotant' fixed the tissue and gave excellent cytological results. After water there was loss of pancreatic zymogen granules and contents of ducts and vessels. Bizarre changes occurred in nuclei, which were absent after isosmotic flotants. Thus, it appeared that frank chemical fixation entailing gross protein denaturation did not accompany freeze-substitution.

Adenopituitary cells examined by refractometry and microincineration. By J. Kruszynski. *University of Liverpool*

It has been shown recently that histological sections may be examined successfully by a refractometric technique. Fixed, but unstained adenopituitary cells when mounted in media of different refractive index (R.I.) could be differentiated optically in the phase microscope into α, β and chromophobes, and their protoplasmic structures revealed.

Optical differences were especially pronounced in media of R.I. 1·50–1·52, or above R.I. 1·56. The R.I. of α, β and chromophobes is different, but it is affected by the fixative; it ranges from R.I. 1·54–1·57. Chromophobes show a much wider range than chromophiles, especially when freeze-substitution technique is used.

Attempts have been made to correlate R.I. with staining reactions (Azan), histochemical tests (PAS), and with the amount of mineral deposits shown by incineration technique. Incinerated chromophiles show remains of their nucleus, nucleolus, protoplasmic granulations and even Golgi structure. Alpha and beta cells display extensive variations in the amount of mineral contents. Beta cells forming small vesicles or invading pars nervosa are particularly rich in minerals. Chromophobes after incineration leave a varying amount of ash. Differences in the amount of mineral ash and in R.I. may reflect stages of cell differentiation and function.

Further studies of autografts of ileum into the bladder in rabbits. By J. JOSEPH and G. A. THOMAS. *Guy's Hospital Medical School, London*

In a previous communication it was noted that a 'patch' graft of ileum into the bladder (i.e. a graft without a blood supply) degenerated and became fibrous tissue lined with transitional epithelium, whereas a 'pedicle' graft of ileum (i.e. with its blood supply intact) maintained its structure after a period of 1–8 weeks. A further series of animals in which the pedicle was tied and cut after 3 weeks was studied. It was found that the ileum grafted on to the bladder retained its characteristic structure after periods of 2, 4, 8 and 16 weeks. Two animals had their pedicles tied and cut immediately after the operation. In these after 4 and 8 weeks a considerable amount of the ileum was replaced by fibrous tissue lined by transitional epithelium. There was evidence that the remainder of the ileum would undergo a similar change. In two rabbits, the pedicles were tied and cut after 1 week and the ileum was found to be intact after 6 and 7 weeks.

In the course of examination of sections of the grafted bladder, it was observed that transitional epithelium in some instances was present on the tips of a few villi near the sutured edge of the ileum and bladder, and had replaced the columnar epithelium which was present on either side. The probable source of this transitional epithelium was discussed.

Further observations on the fate of cartilage grafts in the rabbit, with particular reference to the uptake of labelled sulphate by long term auto-, homo- and heterografts. By M. B. L. CRAIGMYLE. *University College, Cardiff*

Two diametrically opposed views have been expressed as to the fate of the cells in a cartilage graft, the one that they survive and the other that they die after transplantation. Recently, Curran & Gibson (1956) have shown uptake of ^{35}S-labelled sulphate *in vitro* to be peculiar to living chondrocytes. Wyburn & Bacsich (1955) have demonstrated uptake of ^{35}S-labelled sulphate by the cells of homografts of xiphoid cartilage after 21 days in guinea-pigs.

In the present series of experiments two rabbits have borne multiple recoverable autografts of costal cartilage for 659 and 671 days, respectively; four rabbits have borne multiple recoverable homografts of costal cartilage for 659, 667, 671 and 757 days, respectively; but in only one of three rabbits in which multiple heterografts of guinea-pig costal cartilage were sought after 679 days was a single small fragment of cartilage recovered.

Twenty-four hours before recovery of the grafts, each animal was given 5 mc. of $Na_2{}^{35}SO_4$ subcutaneously. After formalin fixation and paraffin embedding, autoradiographs were performed on 7μ sections of each graft using the stripping film technique. Other sections were stained by haematoxylin and eosintoluidin blue, Alcian blue and the PAS reaction. Every graft recovered gave a positive autoradiograph with activity localized over the cartilage cells. The histochemical techniques employed showed the matrix of every graft to respond in a practically normal manner. It is thus concluded that the grafts consisted of viable cartilage.

Adrenal medullary grafts in the rat. Factors affecting the formation and release of pressor amines in grafted chromaffin cells. By R. E. Coupland. *University of Leeds*

Auto- and homografts of rat adrenal medulla have been prepared in the anterior chamber of the eye. No evidence of proliferation of chromaffin cells has been obtained in either animals with one adrenal intact or in completely adrenalectomized animals; no mitotic figures have been observed even after colchicine administration. As reported by Carlsson & Hillarp, reserpine in a dose of 5 mg./kg. (intravenous) completely depletes the rat adrenal of pressor amines. If a graft is made from a completely depleted adrenal gland a chromaffin reaction is apparent within 7 days of operation and is well marked by 14 days. This indicates that pressor amines can be synthesized by completely denervated chromaffin cells. Insulin is without effect on grafted chromaffin cells, but reserpine produces a reduction in the chromaffin reaction of these elements. The results obtained after the application of the chromaffin and potassium iodate reactions to both grafted and intact adrenal medullae in normal, reserpine- or insulin-treated animals support the concept that the adrenaline and noradrenaline secreting cells are distinct entities.

The caput epididymidis and trypan blue. By E. W. Macmillan. *University of Liverpool*

By means of a technique previously described (Macmillan & Harrison, 1955) 0·5% trypan blue solution in normal saline was introduced directly into the vasa efferentia in a random series of mature rats. Specimens examined after sacrifice at periods varying from 24 hr. to 2 weeks after operation showed a staining, clearly visible to the naked eye, of the vasa efferentia and of the immediately adjoining initial segment of the caput. These portions of the duct system of the testis were distinguishable histologically from the ductus epididymidis proper and under normal circumstances were practically empty of spermatozoa.

The macroscopic appearances showed a striking delimitation of the initial segment from the unstained adjacent lobules of the head, despite the fact that the main mass of dye injected could be followed at intervals along the ductus epididymidis towards the exterior, reaching the tail-body junction four days after operation.

The findings, which were briefly discussed, were additional to those of other workers, e.g. von Möllendorf (1920), von Lanz (1926) and Young (1933) who employed subcutaneous injection of the dye, and Mason & Shaver (1953) who introduced the dye just deep to the tunica albuginea of the testis.

The role of mucosal apposition in urinary and faecal control. By S. A. Vincent and G. D. F. McFadden. *Anatomy Department, Queen's University, Belfast; Belfast City Hospital;* and *Ulster Hospital for Children and Women*

A number of different operations aimed at strengthening the pelvic floor have been devised in the past in the attempt to relieve incontinence of urine and faeces. Recently some of our patients here have been cured of enuresis and of incontinence of faeces by operations which were aimed at producing mucosal apposition at the bladder neck and at the ano-rectal junction without attempting to strengthen the pelvic floor. In view of this it was considered that mucosal apposition may be a vital factor in normal control of the bladder and rectum, a conclusion which was supported by cysto-urethrography which demonstrated lack of apposition in cases of urinary incontinence (e.g. caused by distal obstructions or paralysis of the pelvic musculature).

Further evidence was obtained from experiments carried out on fresh post-mortem bladders subjected to varying internal fluid pressures. The external pressure at the bladder

neck required to prevent outflow from the bladder was measured and found to be surprisingly small in relation to the internal vesical pressure. The results support the view that mucosal apposition, requiring very little effort, is a very important factor in maintaining normal control of the bladder (and probably of the rectum also).

The tendons of flexor digitorum profundus. By B. F. Martin.
University of Sheffield

It is generally stated that the tendons of the flexor digitorum profundus are oval in shape, but no comment is made upon any change of form of these tendons as they pass through the tunnels produced by the division of the flexor digitorum sublimis tendons.

From an examination of tendons removed both from dissecting room and from post-mortem specimens, it has been found that the flexor digitorum profundus tendons do undergo a change of shape as they pass through the sublimis tendons, though they are oval prior to entering and also after leaving the tunnels. The portion of the profundus tendon that passes through the sublimis tendon is accurately moulded to conform to the varying shape of the tunnel. This localized moulding of the profundus tendon is brought about by changes in direction of its constituent fibre bundles, a feature which can be noted on macroscopic examination and confirmed by 'shredding' the tendon. Microscopic examination of the tendon yields further information on the fibre bundle arrangement.

In the foot, the tendons of flexor digitorum longus are found to undergo the same changes of form when passing through the tendons of flexor digitorum brevis as those undergone by the flexor digitorum profundus in the hand.

A photographic method of graphic reconstruction. By A. D. Dixon and P. Howarth. *University of Manchester*

A technique making use of photomicrographs has been devised to produce accurate three-dimensional reconstructions of fine structures such as nerve plexuses, customarily represented in a purely diagrammatic form.

Photomicrographs are prepared on 35 mm. film from serial sections of tissue orientated by means of small holes drilled through the block before sectioning. Photographic enlargements to the required magnification are then prepared and the nerve fibres inked in with waterproof ink, referring, where necessary, to the original tissue section. The photographs are then bleached out, leaving an inked record of nerve fibre pathways.

Copies of these drawings are made on process plates, the camera being placed at an angle of 45° to the plane of the original; the effect being to reduce the latter in one diameter.

The resulting slightly distorted photographs are positioned serially on parallel lines, which correspond to the drill holes, due allowance being made for the thickness of the sections, and traced on to a superimposed transparent sheet.

The method has the advantage of producing at relatively low cost, an accurate three-dimensional reconstruction, and at the same time a permanent photographic record of all the planes of section.

INDEX TO VOLUME 91

SUPPLEMENTARY INDEX OF PROCEEDINGS